Reproductive Technologies Applied to Newfoundland Salmonid Aquaculture to Enhance Commercial Production

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by

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ABSTRACT

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A long-term goal of the Newfoundland Salmonid Growers Association (NSGA) has been to use Atlantic salmon (*Salmo salar*) brood stocks that are adapted for superior performance under Bay d'Espoir aquaculture conditions. As part of this aspiration, the association undertook to evaluate technology for the production of all-female and reproductively-incapacitated strains of Atlantic salmon. Aquaculture businesses are interested in pursuing technology to improve cost effectiveness and profits from salmonid farming. DFO is interested in pursuing further evaluation of methods for the production of non-maturing salmonids as a means to avoid concerns about potential long-term genetic consequences of breeding of escapees from marine cages with wild salmon of the Bay d'Espoir area. This provided a common ground for evaluating reproductive technologies for Bay d'Espoir aquaculture.

Variability in production characteristics of triploid salmon from year to year and susceptibility to local pathogens proved to be a concern to the salmon-farming industry. A greater incidence of jaw deformities observed among triploid Atlantic salmon appeared inconsequential as the deformity did not seem to affect performance and all of the salmon were marketed as fillets. Proximate analyses failed to identify differences between diploid and triploid fillets that might influence consumer acceptance.

This report describes Newfoundland aquaculture-industry efforts towards establishing all-female, diploid and all-female, triploid lines of Atlantic salmon in support of Bay d'Espoir aquaculture during the interval of 1992 to 2000. Highly influenced by cash-flow challenges, insufficient hatchery facilities to carry experimental lines, and the interpretation of greater susceptibility of triploid salmon to the fish-pathogen spectrum of the Bay d'Espoir estuarine fjord, the Bay d'Espoir industry terminated its pursuit of Atlantic salmon reproductive-technologies research in 1998. Overall the main conclusion arising from a decade of experience is that a triploid Atlantic salmon produced from a superior-performing aquaculture strain can result in performance advantages. The range of environmental conditions under which superior performance can be expected remains unresolved.

RÉSUMÉ

Pepper, V.A., Nicholls, T., and Collier, C. 2004. Reproductive technologies applied to Newfoundland salmonid aquaculture to enhance commercial production. Can Tech. Rep. Fish. Aquat. Sci. 2541: v + 50 p.

La Newfoundland Salmonid Growers Association (NSGA) a eu comme objectif à long terme d'utiliser des stocks de saumon atlantique (*Salmo salar*) reproducteurs adaptés pour donner un rendement supérieur dans les conditions d'aquaculture propres à la baie d'Espoir. À cette fin, l'association a entrepris d'évaluer la technologie de production de souches de saumon atlantique composées exclusivement de femelles rendues inaptes à la reproduction. Les entreprises d'aquaculture s'intéressent aux progrès technologiques susceptibles d'accroître le rapport coût-efficacité et les profits de l'élevage du saumon. Pour sa part, le MPO cherche à évaluer plus avant les méthodes de production de salmonidés qui n'atteignent pas la maturité comme moyens d'éviter les risques de répercussions génétiques à long terme découlant de l'accouplement d'évadés des cages de mariculture avec du saumon sauvage de la baie d'Espoir. Il y avait donc là un intérêt commun pour l'évaluation de technologies de reproduction pouvant s'appliquer à l'aquaculture dans la baie d'Espoir

La variabilité des caractéristiques de production de saumons triploïdes d'année en année et la vulnérabilité aux pathogènes locaux se sont avérées des sujets d'inquiétude pour l'industrie de l'élevage du saumon. Une plus grande incidence de difformités de la mâchoire ne semble pas avoir eu de conséquences, car elle était apparemment sans effet sur le rendement et que tout le saumon était commercialisé sous forme de filets. Les analyses immédiates n'ont pas dégagé de différences entre les filets des saumons diploïdes et ceux des saumons triploïdes susceptibles d'influencer l'acceptation par le consommateur.

Le présent rapport fait état des efforts déployés de 1992 à 2002 par l'industrie de l'aquaculture de Terre-Neuve pour constituer des lignées de saumons atlantique diploïdes et triploïdes composées uniquement de femelles et destinées à soutenir l'aquaculture dans la baie d'Espoir. En grande partie à cause de problèmes de liquidités, d'un manque d'écloseries pour produire les lignées expérimentales et de l'interprétation d'une plus grande vulnérabilité du saumon triploïde au spectre de pathogènes du poisson du fjord estuarien de la baie, l'industrie de l'aquaculture de la baie d'Espoir a mis fin à ses recherches sur les technologies de reproduction du saumon en 1998. Dans l'ensemble, cette décennie d'expérience permet de conclure qu'un saumon atlantique triploïde provenant d'une souche aquacole à rendement supérieur peut procurer des avantages pour la production. On ne sait cependant pas quelle est la gamme de conditions environnementales dans lesquelles on peut escompter un rendement supérieur.

INTRODUCTION

Economically-viable salmon and trout aquaculture requires use of species that grow well under the confined and artificial conditions of hatcheries and rearing cages. In addition, these species must be highly tolerant of human intervention in the daily activities of the fish. This is in marked contrast to wild species that must be extremely wary to survive. A challenge for finfish aquaculture is to enhance species characteristics that are desirable for artificial rearing and to suppress undesirable traits. Accordingly, finfish that are used in an aquaculture business, while they may be the same species as wild fish, typically must be manipulated and selected to perform well under intensive fish-culture conditions.

Sustainable development of Canadian aquaculture requires careful attention to biological prerequisites, concurrent with consideration of economic constraints for successful competition in international seafood markets. With its dual mandate as both a regulator and enabler of Canadian aquaculture, Fisheries and Oceans Canada is obliged to examine aquaculture practices in the context of minimizing risk to wild aquatic resources. As noted in DFO's Aquaculture Policy Framework, this mandate obligation is addressed in a client-centred approach based on partnering with industry, other agencies and the public. Much of DFO's aquaculture mandate is accomplished through close working relationships with industry participants to assure ongoing, two-way communications regarding aquaculture-development issues. This operations model has been embraced by the Newfoundland Salmonid Growers Association (NSGA) that has been anxious to pursue salmonid farming research that would serve the dual purpose of improving production efficiency and furthering environmental stewardship.

Industry is interested in pursuing technologies to improve the cost effectiveness of salmonid farming. A long-term goal of the NSGA is to identify and adopt brood stocks and husbandry practices to achieve superior salmonid performance under aquaculture conditions of the Southwest coastal area of the island. The salmonid farmers began their investigations of alternative technologies for broodstock management in1985, partly due to an ongoing need for increasing efficiencies in the business of aquaculture, and partly due to regulatory requirements of DFO.

There are potential strategic, economic, and environmental advantages to using late-maturing or non-reproductive salmonids for aquaculture operations. Elimination of male salmonids from aquaculture strains is one strategy for reducing the incidence of early-maturation and for extending the market window for fresh aquaculture product. Imposed retention of all maternal chromosomes following egg fertilization with normal salmon sperm (i.e. triploidy) is one method to overcome the normal reproductive cycle. However, the triploidy approach is confounded by the fact that, unlike females, male triploid salmon do undergo gonadal development (Arai 2001). Although resulting spermatozoa are not viable, male triploids show the same physiological diversion of energy into reproduction as normal-diploid males and therefore, reduced growth performance. Spermatozoa of triploid-male salmon are capable of activating but not sustaining egg development. This has the advantage that any salmon that might escape

from aquaculture cages would not have a genetic influence on wild salmon stocks due to an inability to reproduce. However, Kitamura et al. (1995) suggested that diploid female masu salmon (*Oncorhynchus masu*) could spawn with a triploid male even in a natural environment. Given that triploid males do demonstrate spawning behaviour, successful competition for spawning opportunity by such males represents a potential disaster to wild populations since any eggs activated by such males would die (Thorgaard and Allen 1987).

Fishery resource managers have been concerned about potential reproductive interactions between escapees from salmon aquaculture cages and natural spawners in Newfoundland rivers. Scientists have cautioned about preservation of genetic diversity of wild salmonid populations (Cloud and Thorgaard 1991). Such concerns have resulted in international agreements on Atlantic salmon (*Salmo salar*) conservation (Anon. 1994) that have encouraged adoption of technology for reproductive control of Atlantic salmon by the aquaculture industry. Amid such concerns about potential negative genetic consequences of interbreeding between aquaculture and wild salmon, the Newfoundland and Labrador Region, Science Branch was directed in 1989 to investigate alternative means to impose reproductive dysfunction among aquaculture species. DFO is interested in pursuing development and evaluation of biotechnology applications to salmon husbandry for the production of non-maturing salmon as a means to avoid concerns about potential long-term genetic consequences of interbreeding of escapees from marine cages with wild salmon of the Southwest Coast of the island. For industry, potential interest in means of achieving reproductive dysfunction of aquaculture salmon is to avoid food-energy diversion away from somatic growth and into gonad development.

An Atlantic Canada workshop (Pepper 1991a) provided insight into methods for the production of non-reproductive Atlantic salmon for aquaculture. On the basis of the knowledge gained from that forum (Jungalwalla 1991), and a subsequent discussion of the matter with Newfoundland industry representatives (Pepper 1991b) a research program was designed to develop a line of all-female Atlantic salmon for Newfoundland aquaculture and to evaluate the performance of triploid Atlantic salmon under Newfoundland aquaculture conditions. Research with this technology in Newfoundland began with rainbow trout (*Oncorhynchus mykiss*) in 1985 (Sutterlin and Collier 1991). Performance evaluations with Atlantic salmon continued through to the fall of 2000.

Considering the potential undesirable consequences of male triploid salmon on wild populations, and inferior male performance in marine cages, this report describes Newfoundland experience with both gender manipulation and triploid performance by Atlantic salmon in Newfoundland marine cages.

GENDER MANIPULATION

Feed-Mediated Hormone Treatment of Alevins

A common phenomenon among salmon in aquaculture is early sexual maturation, a physiological process that often is disproportionately expressed among males. Hence, about half of the salmon of a mixed-sex aquaculture production line often are subject to lower profitability than the female component of the inventory. All-female salmonid strains have the advantage in aquaculture of greatly reduced incidence of grilsing. Application of monosex technology to aquaculture strains has become common practice world-wide and is increasing in popularity. Manipulation of genetically-female salmon alevins and parr to modify endocrine system development in favour of the male phenotype has been applied increasingly to achieve all-female strains of aquaculture fish (Sower et al. 1984; Billard 1989; Herman and Kincaid 1991; Johnstone and MacLachlan 1994; Quillet and Chevassus 1994; Feist et al. 1995; Donaldson et al. 1996; Johnstone 1996; Pepper et al. 1996). Applied only to future brood stock, this hormone-exposure approach is not of concern regarding residues among aquaculture production salmon. As of the early 1990s, aquaculture production of monosex female chinook salmon in British Columbia exceeded the total commercial catch of wild and hatchery-produced chinook on the Pacific coast of North America (Donaldson et al. 1996).

The salmonid endocrine system is poorly differentiated at the alevin, fry and early parr stages and is subject to manipulation by exposure to external sex steroids (Donaldson et al. 1992; Johnstone and MacLachlan 1994; Pandian and Sheela 1995). A mixed-sex group of salmonids, if exposed at the alevin stage to male hormone (androgen) will continue genetically as a mixed-sex population but will be functionally all-male. Treatment with male hormone has no apparent effect on genetic males but, when applied to females during the labile period of gonad differentiation (i.e. about two weeks after hatch), produces a fish that is genetically female but functionally male. At maturity, these genetically female, yet phenotypically male salmonids will produce sperm that is homogametic (i.e. has no male genetic component) and therefore, when used to fertilize normal eggs, will result in all-female offspring. The steroid-treatment technique for producing monosex female salmon has been applied to Bay d'Espoir brood lines (Fig. 1).

A disadvantage of the steroid-treatment approach to gender manipulation is that it requires two generations (at four years per generation) to produce a harvestable all-female population. Even then, all of the fish used in the breeding program must be killed for detailed gonad examination to assure elimination of genetic males. An alternative technique, that results immediately in an all-female brood population, would simplify hatchery logistics and reduce the demand for tank space (i.e. the space that would be required to raise genetically-male salmonids to the time when gonad characteristics are visually apparent). In pursuit of this goal, gynogenesis was adopted as a means by which all-female hatchery lines might be developed with minimal hatchery-space and time-delay requirements.

Gynogenesis

A more direct approach to establishing an all-female strain of salmon is gynogenesis in which spermatozoa are genetically incapacitated by ultraviolet irradiation (Chourrout, 1982). Gynogenesis is a parthenogenic process in which all genetic material is maternally inherited. Unlike the hormone-mediated approach, gynogenesis does not require sex-reversal to produce an all-female strain. Given that spermatozoan DNA actually is destroyed during the UV treatment, the only concern that must be addressed for sperm donors is spermatozoa motility after irradiation.

UV Irradiation

With the gynogenesis approach, sperm is genetically incapacitated by UV irradiation to destroy its DNA (Fig. 2). Such incapacitated spermatozoa are still motile immediately after successful treatment and can activate egg development. Without the normal haploid complement of chromosomes, irradiated spermatozoa can not actually sustain zygote development due to failure of the cell division process (i.e. unpaired chromosomes). However, when combined with high hydrostatic pressure shortly after egg activation, to retain the second set of maternal chromosomes that normally would be eliminated shortly after fertilization, salmon eggs activated with genetically-incapacitated spermatozoa can develop normally and result in progeny that are entirely maternally generated. Such progeny are all-female. However, survival of gynogens through hatch and early development typically is lower than for eggs of normal brood stock (Crandell et al. 1995). It is important to note that the gynogenesis approach to achieving an all-female line is applied only to potential future brood stock. Given the effort involved, and lowered incubation survival, the gynogenesis approach is not applied to production fish. If alevins resulting from the gynogenesis process are placed in a water bath of male hormone as described above to modify early development of the endocrine system, the all-female line will develop as phenotypic males which, when crossed with genetic females at maturity, result in all-female progeny. This approach has the advantage over that applied at Bay d'Espoir in the early 1990s of generating an all-female brood line in one generation without having to eliminate genetic males manually after the expensive on-growing process.

TRIPLOIDY

Techniques for inducing triploidy in salmonids have been reviewed by Benfey et al. (1988). While heat shock has been successful with rainbow trout, efficiency of this technique in early applications to Atlantic salmon was not entirely satisfactory (69%; Sutterlin and Collier 1991). Experience to date indicates that pressure is the preferred means to produce triploid salmonids (Benfey et al. 1988; Johnstone et al. 1991). The correct timing for administering pressure shock to fertilized eggs is temperature dependent and critical. Protocols for inducing triploidy are well established for rainbow

trout and have been documented as well for Atlantic salmon (Jungalwalla 1991; Benfey et al. 2000).

For several years, industry personnel and researchers have been studying the possibility of using non-reproductive Atlantic salmon for aquaculture purposes. Industry's main interest in this technology is motivated by deteriorating flesh quality among maturing fish along with increasing (deteriorating) food conversion ratios. With the Atlantic salmon industry in Bay d'Espoir, early maturation typically is <10% but can exceed 50% in a given production unit (Pepper 2003). These fish become unmarketable due to changes in appearance and flesh quality (Lincoln and Scott 1983, 1984; Solar et al. 1984; Beacham and Murray 1986; Bye and Lincoln 1986; Donaldson and Benfey 1987; Benfey et al. 1989; Seeb and Miller 1990; Johnstone et al. 1991; Galbreath et al. 1994). A non-maturing strain of production salmon would improve industry economics. However, in some settings, triploid fish have demonstrated inferior performance relative to normal diploid fish, especially when grown together (Galbreath et. al. 1994).

Since 1989, Department of Fisheries and Oceans researchers in Newfoundland have been working to evaluate methods to produce non-reproductive Atlantic salmon for aquaculture. The reproductive-technologies research evolved to include comparisons of growth and survival rates of these non-reproductive Atlantic salmon with those used as normal production for the commercial aquaculture industry.

Background to this report is found in Pepper (1991a, 1991b). This document describes a decade of Newfoundland experience in gender manipulation to produce all-female lines of Atlantic salmon, application of pressure shock for generation of triploid salmon, and the relative performance of resulting livestock.

METHODS

SALMON GAMETE PROCUREMENT

Brood salmon were maintained in cages at industry production sites of the Bay d'Espoir estuarine fjord until early fall of the year in which spawning was expected. Some weeks in advance of expected spawning time, brood cages were towed to Ship Cove (Fig. 3), an estuarine site in Bay d'Espoir where there is a significant freshwater surface layer. As spawning time approached, normal female brood salmon were checked periodically to ascertain their behaviour (i.e. depth in the cage) and stage of maturation. Once spawning was imminent among normal brood salmon in the estuarine cages, presumed sex-reversed fish were killed with an overdose of anaesthetic. These specimens were washed thoroughly to remove anaesthetic residues, dried with clean paper towel, and then dissected on site to observe the gonads.

GENDER MANIPULATION

Feed-Mediated Hormone Treatment of Alevins

As reported previously (Pepper et al. 1996), all Newfoundland efforts towards Atlantic salmon gender manipulation prior to 1995 were conducted with a mixed-sex strain. This early experimentation with sex reversal was based on a steroid treatment using 300 μg of 17α-methyltestosterone (MT)·kg^{-1} of feed from first-feeding of alevins for an interval of 500 C° days. All of these hormone-treated specimens were retained at the hatchery (fresh water) throughout their life history.

Immersion Treatment

Starting in 1995, the Bay d'Espoir industry began experimenting with immersion treatment of alevins in a synthetic-androgen bath. Endocrine system development of genetically-female salmon alevins was manipulated by immersion treatment at 50% hatch + 7-14 days at 10°C. Batches of 5000 alevins (determined volumetrically) were subjected to a bath of 17α-methyldihydrotestosterone (MDHT) at a concentration of 2,000 μg·$litre^{-1}$ at 250 alevins per litre. Alevins were immersed in the controlled-temperature bath for 1200C°minutes, after which they were flushed with incubation water and returned to the incubation tray to continue development prior to being placed in hatchery tanks. The detailed immersion-treatment protocol is presented in Appendix 1.

An alternative protocol, motivated largely by Piferrer et al. (1993), also was applied in an attempt to determine relative efficacy. In 1999, alevins were subjected to two 2.5 hour-immersion treatments of MDHT @2000mg·l^{-1} administered at 7°C, the first at 10 days and the second at 18 days after 50% hatch. Contrary to the treatment applied to the 1998 year-class, in which only a single bath was applied, the "double-dipping" experiment undertaken in 1999 was intended to address whether the incidence of hermaphrodites observed in 1997 could be improved with a second priming of the endocrine system, or if this additional exposure might evoke sterility.

To facilitate comparison of immersion treatments with results of the protocol applied in previous years, a third group of presumed all-female fry was set aside for MDHT treatment at the time of first feeding as per the previous feed-mediated protocol to establish an all-female line. For all of these potential brood salmon, on completion of their freshwater (hatchery) phase, these presumed sex-inverted salmon were grown to maturity in broodstock cages in the Bay d'Espoir fjord.

Gynogenesis

The first Newfoundland attempt at gynogenesis with Atlantic salmon in November 1996 was based on protocols worked out for rainbow trout. These protocols (Palti and Thorgaard 1997) were adopted for gynogenesis experimentation on Atlantic salmon in Bay d'Espoir. Ultraviolet light was supplied by a standard germicidal lamp

(Rainbow Lifegard QL-40) with a wavelength of 2537 angstroms. The light fixture was mounted on a stand and the distance to the sperm container (~40 cm.) adjusted to provide an intended exposure of 20ergs·mm^{-2}·sec^{-1}.

Essential to effective application of the Palti and Thorgaard (1997) protocol is a quartz crystal container for the sperm that would allow accurate measurement of the UV dosage. Unfortunately, even with much effort to procure such containers in 1996, they did not arrive in time for this first experiment. Thus, for this first trial exposure, sperm was placed in a Pyrex® baking dish for irradiation. UV light intensity was measured with a shortwave ultraviolet meter (Ultraviolet Products Model J-225) placed immediately beside the dish. Total exposure was targeted at 12,000 ergs·mm^{-2} surface area at a total exposure time of 10 minutes.

The 1996 experiment utilized eight incubation trays of ~8000 eggs each. This experiment consisted of two trays diploid control (normal sperm), two trays mixed-sex triploids (normal sperm, pressure-treated zygotes); and, four trays of gynogenesis eggs (UV-irradiated sperm, pressure-treated zygotes), for a total of some 64,000 eggs in anticipation of an analysis of incubation success of 'k independent samples'. Incubation trays were monitored and dead eggs counted and removed once per week during the nine-week incubation period. Most eggs hatched during the week of January 20. On January 21/97, the two trays of mixed-sex triploid alevins were eliminated from the experiment to avoid potential future concerns regarding maturing male triploid salmon and associated spawning behaviour. Approximately 2,000 of the presumed gynogenic alevins were removed from one incubation tray and treated with MDHT to effect sex-reversal. Presumed gynogenic juveniles from this experiment were ponded in the Bay d'Espoir hatchery and grown until they were large enough (5 - 10 g) to allow blood sampling for flow-cytometry analysis.

UV exposures were applied to 25 ml of 10% extender-diluted sperm solution. For all sperm lots that received extender solution, spermatozoa were examined for motility both before and after activation with water. The group that received ultraviolet irradiation also was examined for motility after treatment.

Unfortunately, among juveniles resulting from the gynogenesis approach using conspecific sperm, there was no easy visual way to distinguish gynogenic parr from the normal, mixed-sex parr. Overexposure of sperm will kill spermatozoa and result in failure to activate egg development. Under-exposure can leave spermatozoa essentially untreated, thereby resulting in mixed-sex zygotes. A revised experimental protocol was applied in November, 1997 (using the proscribed quartz irradiation dish), when brook trout (*Salvelinus fontinalis*) sperm was irradiated with three different dosages of UV-C (15,000; 17,000; and 19,000 ergs·mm^{-2}) and used to activate salmon egg development. The newly-activated salmon eggs were subjected to pressure shock (9,500 lb·in^{-2} at 300C°minutes after activation for a period of 5 min) to impose retention of all of the maternal chromosomes. As Atlantic salmon female x brook trout male crosses are not naturally viable, any alevins produced from this gynogenesis process must be of complete maternal inheritance. Batches, totalling ~108000 salmon eggs were treated in this

fashion on November 11, 1997 and placed in 15 incubation trays. Dead eggs were removed routinely throughout the incubation interval, counted and the data recorded at the incubation site.

This same technique was applied again in November of 1999 under the more controlled laboratory conditions of the Northwest Atlantic Fisheries Centre (NAFC) in St. John's. For this experiment, Atlantic salmon eggs were collected from the industry's brood stock in Bay d'Espoir and transferred to St. John's in sealed containers (large surface-area-to-volume ratio) on slush ice. Brook trout sperm was obtained from a certified, disease-free hatchery in Quebec. For this experiment, six trays of ~2500 eggs each (determined volumetrically) were used as follows: three trays of control-group eggs (normal fertilization) and three trays of UV-treated, pressure-shocked eggs. As per the protocol of Appendix 1, 22 days after modal hatch, two of the trays of alevins (1 gynogenesis, 1 control) were subjected to immersion treatment with MT and two were subjected to immersion treatment with MDHT. All groups were retained and monitored through the incubation and subsequent life-history stages at the NAFC. All surviving specimens were sacrificed and dissected on completion of the rearing interval (March, 2003). This final trial of the gynogenesis protocol was undertaken in an attempt to determine if the process could be more successful under laboratory conditions than when facing the real-world constraints of industry production facilities.

Homogametic sperm

In October of 1997, a cull of 51 potentially homogametic salmon of the 1995 year class from the marine cage at Strickland Cove was examined for evidence of sex-reversal. Only three hermaphroditic gonads were found. 77% of this group of presumed sex-reversed salmon were considered immature. These potential brood salmon then were towed to Ship Cove (greater freshwater influence) for final maturation. In November of 1997, all of the 1994 year-class specimens of androgen-treated salmon and 30 specimens of the 1995 year class were sacrificed and dissected to determine incidence of sex-reversal. On the basis of visual examination of the gonads, the observation of paired and well-formed ovaries was considered definitive evidence of failure of the sex-reversal process. Presence of at least one well-formed testis is definitive only if the sex-reversal technique was applied to a known group of all-female salmon. In the case of hormone treatment of mixed-sex alevins, a well-formed testis was considered indicative of a genetic male. A seriously-deformed testis may or may not be a successfully masculinized female.

For purposes of this study, only those specimens visually identified as hermaphroditic (i.e. an individual with an obvious ovary and a well-formed testis; Fig. 4) were accepted as homogametic. The testis component of the gonad was excised from the hermaphroditic specimen, then placed in a sealed, air-filled container on slush ice and transferred to the industry's incubation facility (< 0.5 hour transfer time). In preparation for egg fertilization at the incubation facility, an individual homogametic testis was placed in a pouch of dry, fine mesh material (commercial panty hose), scored sufficiently

to disrupt the tissues, and then placed in a domestic potato ricer. The gonad was squeezed gently so that the sperm oozed into a clean, dry beaker. All homogametic sperm samples were checked for spermatozoa activity by observation under a microscope (400X) while a small drop of fresh water was added to the microscope slide to activate spermatozoa. The extracted sperm from obviously sex-reversed fish was mixed with a modified Cortland's sperm-extender solution (Disney et al. 1987), placed in separate storage containers with large surface-area-to-volume ratio and stored in a domestic refrigerator until use (i.e. typically less than four hours).

In parallel with hermaphrodite sampling, eggs from normal brood females also were transferred in insulated containers on slush ice to the industry's incubation facility. Good quality eggs were transferred back to the hatchery where a random sample of some 175,000 eggs (subsets from about 100 brood females) was fertilized with presumed homogametic milt from the sex-reversed salmon dissected earlier in the day. When it was time to fertilize eggs, aliquots of 700 ml of green eggs were measured into modified two litre graduated cylinders and then placed into a plastic pan. These pans were allowed to float in a bath of incubation water for several minutes before use to equilibrate temperature to that of the incubation unit (8°C.). The breeding scheme (Pepper and Crim 1996) was intended only to "homogenize" genetic variability among the experimental groups. Eggs of either the all-female or all-female-triploid groups were incubated in parallel with the industry's production line (i.e., mixed-sex, diploid) of salmon. All batches of eggs were enumerated volumetrically and surface disinfected with iodophor. After surface disinfection, incubation trays were labelled as to treatment group and placed in vertical-drip incubation stacks .

TRIPLOIDY

By 1995, we had developed a line of presumed all-female, non-reproductive Atlantic salmon (experimental group) for performance evaluation in parallel with a group of normal, reproductively-viable salmon of the same strain (control group). The procedures and apparatus used for preventing elimination of the second set of maternal chromosomes during early cell division in Atlantic salmon were as described by Pepper et al. (1996; Proceedings of the Special Session on Use of All-female and Triploid Salmonids for Aquaculture in Canada). As a result of discussions and suggestions from that mid-1995 forum, we modified our earlier protocol for handling eggs prior to, during and immediately after pressure shock in an attempt to reduce handling stress on newly-fertilized eggs. Refinement to the post-fertilization egg-handling process involved development of a miniature upwelling incubation system based on 1.5 litre graduated cylinders. This apparatus was designed and assembled (Fig. 5) to act as an interim up-welling incubator while the eggs were going through the water-hardening process. Each 700 ml batch of fertilized eggs was put into one of the plastic graduated cylinders. These were placed into receptacles in the water-hardening bath and connected to a central water-supply manifold via flexible plastic tubing. Water was circulated though the manifold and into the bottom of each graduated cylinder via this tubing with a Little Giant® pump. All cylinders were surrounded by incubation water in this bath to

stabilize temperature. This simple refinement to the post-fertilization egg-handling process allowed us to place discrete batches of eggs (i.e. equivalent to the capacity of one incubation tray) directly into the pressure chamber with minimal handling.

Pressure treatment was applied to eggs fertilized with homogametic milt. Fertilization, pressure treatment and surface disinfection all took place at the hatchery within hours of procuring eggs from the marine cages. After the required 300 C°-minute interval following fertilization, each cylinder was placed into the pressure chamber (Fig. 6) and pressure applied at 9500 psi for 5 minutes. The cylinders of eggs then were returned to the recirculating water bath and left to swell for a minimum of two hours. Following the water-hardening interval, eggs were surface disinfected with 100 $mg \cdot L^{-1}$ Ovadine (Syndel International Inc.) in a 10-minute static bath and then flushed with fresh water for one hour.

Concurrently, industry personnel fertilized eggs for the annual production requirements with milt from normal male brood fish. Eggs were incubated at 8°C. Thus, there were three sets of salmon eggs laid down in the Bay d'Espoir hatchery in 1995 (i.e. to produce the 1996 year class): 1) mixed-sex diploids; 2) all-female diploids; and, 3) all-female triploids. The first picking of dead eggs from the incubation trays occurred about eight hours after fertilization. Eggs were addled at the eyed stage and dead eggs removed.

Experimental groups of salmon fry were screened for ploidy status by erythrocyte smears (pre 1995) and by flow cytometry (post 1994). Immediately prior to blood sampling, salmon fry were netted and anesthetized quickly with 2-phenoxyethanol. These fry were bled by caudal puncture using 26 gauge hypodermic syringes. Syringes were flushed with sodium heparin to prevent clotting. Samples of 3-4 µl of whole blood were added to 2 ml of propidium iodide solution (i.e. 50 $mg \cdot L^{-1}$ propidium iodide in 0.1% sodium citrate) in a disposable polystyrene tube that fit into the flow cytometer. All samples were taken at the Bay d'Espoir hatchery, placed in pre-labelled tubes, agitated with a vortex blender, and stored on slush ice for about 24 hours, during which time they were transported to the School of Medicine (Memorial University of Newfoundland) in St. John's for flow cytometry analysis. Flow cytometry was performed using a FACSTAR PLUS flow cytometer (Becton Dickinson, CA).

Approximately 175,000 all-female salmon eggs were fertilized with homogametic milt in 1997, treated with pressure shock and then placed in the St. Alban's incubation facility in November. Triploid fry of the resulting 1998 year-class were transferred to tanks in the Bay d'Espoir hatchery in February of 1998 and were retained there until October 26. As hatchery space was at a premium throughout the rearing interval for this year class, these triploid salmon parr were kept in four, 1.2m diameter ($0.45 m^3$) tanks where they reached a maximum density of 68 $kg \cdot m^{-3}$.

In support of demonstration of comparative performance of locally-produced, triploid Atlantic salmon relative to those of a commercial supplier of an alternative strain (Cascade AquaFarms, Gaspé strain), a local Bay d'Espoir company imported 50,000

triploid eggs in late December 1997. Resulting fry were placed in two, 1.2 m diameter tanks in the hatchery February. Gaspé strain triploid parr were top graded (15 mm grader) in July and transferred to a single 9.8 m^3 tank in the Bay d'Espoir hatchery in August where they achieved a maximum density of 26 $kg \cdot m^{-3}$.

The various initiatives described above are summarized in Table 1.

Table 1. Gender and ploidy manipulation experiments applied to Bay d'Espoir aquaculture.

Year Class	Goal	Treatment
1994	Homogametic sperm	Feed-mediated MT
1995	Homogametic sperm	Single immersion, MDHT
	Triploidy	Pressure
1996	All-female zygotes	Gynogenesis: salmon sperm, pyrex chamber
1997	All-female zygotes	Gynogenesis; brook trout sperm, quartz chamber
	Homogametic sperm	Single immersion, MDHT
	Triploidy	Pressure
	Triploidy	Importation, Gaspé strain triploid eggs
1998	Homogametic sperm	Single immersion, MDHT
1999	Homogametic sperm	Double immersion, MDHT
	All-female zygotes	gynogenesis
2000	Homogametic sperm	Single immersion, MT
		Single immersion, MDHT

AQUACULTURE PERFORMANCE

Husbandry

Husbandry practices throughout the duration of these experiments were those applied by industry according to their business practices and state of knowledge at the time. Accordingly, fish-health management practices evolved during the interval of these experiments. Of significance is the changing fish-health protocols under which the livestock was managed. While antibiotics had been applied prior to 1995, vaccination against the pathogen spectrum of the Bay d'Espoir area was the preferred approach thereafter. Oil-based vaccines (Alpharma, AquaHealth and Micrologix Biotech) were applied for the first time in Bay d'Espoir in 1995. In that year, Atlantic salmon smolts were vaccinated with various oil-adjuvanted vaccines containing antigens specific to vibrioses (i.e. two summer *Vibrio* sp. strains, one winter vibrio) and one strain of furunculosis (*Aeromonas salmonicida*). Vaccination in subsequent years employed primarily AquaHealth Lipogen Triple or Lipogen Forte.

The 1995 year-class salmon were monitored throughout their early hatchery phase. Due to the necessity of a hatchery retrofit to resolve some hatchery logistics problems, these parr were transferred from the hatchery as underyearling parr on December 7 and placed in estuarine cages in the freshwater lens. Sampling prior to

transfer took place in the hatchery on October 26 for the triploid parr and on November 22 for the all-female diploid parr. Samples of this year class of triploid and diploid salmon were taken from marine cages opportunistically during 1996 and 1997. Live weight at each sampling interval was monitored as part of the experimental design. Samples of 35 specimens from each cage were dipnetted during feeding. Typically batches of 1 – 3 specimens were anaesthetised (TMS bath), and weighed and measured individually through the summer months. Salmon were fed daily by industry personnel and records maintained of daily mortality and the weight of feed distributed to each cage each day.

As per normal industry practices at the time, salmon cages were relocated between summer (Strickland Cove) and winter (Roti Bay) farm sites (Fig. 3). In May of 1997, the "control" group of the 1995 year class of industry production fish was combined with 5542 salmon from another cage at Strickland Cove where they were kept through their final summer of marine growth. All of these 1995 year-class salmon were harvested in October, 1997. At the time of processing for market, a random sample of 25 salmon from each group was examined, from which fork length, whole weight, head-on-gutted (HOG) weight, any incidence of physical abnormalities, fillet weight and flesh colour (subjective; normal, pale, etc.) was recorded. One fillet per salmon was frozen for proximate composition (i.e. percent moisture, fat, protein, and ash). Analysis of the amount of pyloric-caeca fat was undertaken to provide insight into the energetics of reproductive vs. non-reproductive salmon physiology. Chemical analysis of carcass quality was done by the Canadian Food Inspection Agency as per procedures of the Association of Official Analytical Chemists (1984).

In the spring of 1997, 11970 all-female triploid Atlantic salmon (1996 year class, SJ strain) were placed in marine cages in Margaree Cove in parallel with comparison groups of 13184 all-female diploids and 12000 mixed-sex diploids. These salmon were maintained at this site through their first summer of on-growing where they were sampled on an approximately monthly basis through the summer. These fish were transferred to the Little Passage site (Strickland Cove) in the spring of 1998 where they were maintained until October 13, at which time they were graded (subjectively by visual appearance) into the usual industry categories of small, large and grilse. Sampling was conducted opportunistically through the summer and again at the time of grading.

Blood samples were secured in September, 2000 to confirm the ploidy status of the Cascade strain of triploid salmon. These blood samples were prepared as described above and transferred to the Memorial University flow cytometry lab in St. John's (~36 hours later) for quantification of DNA content.

Performance-Comparison Analyses

The suite of performance indicators for these reproductive-technology experiments included growth, survival and food conversion ratio (FCR). Data collected

during the experiment were examined for overall patterns of strain performance. Comparison statistics were developed as per Pepper et al. (2003).

Relative performance evaluations focused on mean weight and biomass of the diploid and triploid strains at the beginning and again at the end of the experiment. Instantaneous growth rates (Ricker 1975) between sampling intervals were used to calculate daily mean weight for each strain, which, combined with records of daily mortality and amount of food dispensed, allowed interpretation of daily biomass, growth and feed conversion. Estimates of increase in biomass during the marine on-growing interval were based on biomass calculations using start and end mean weights and start and end inventory. Given that these calculations are based on periodic data from specimen sampling and daily industry records of feeding, mortality and remaining inventory, the disparate data sets of the three year classes of these performance comparisons were limited to the on-growing intervals of complete temporal overlap. Comparison statistics were calculated using STATISTICA™ V6 (StatSoft Inc.). Approximate confidence intervals for estimates of increase in biomass between the start and end of the experiment were determined as per Milliken and Johnson (1984; STATISTICA macro 'confidence intervals.svb') and Sokal and Rholf (1973) using separate variance estimates for the start and end (harvest) populations.

RESULTS

SALMON GAMETE PROCUREMENT

Motility of spermatozoa after dissection of sex-reversed brood fish generally was low relative to that for wild salmon. In fact, had there been any options, some of the lots of homogametic milt would have been discarded. Interestingly, after storage in the sperm extender and subsequent activation with water, spermatozoa activity increased. Samples of eggs taken at intervals following placement in incubation trays confirmed that fertilization had taken place. With the exception of the egg take in 1997, for which there was a high incidence of over-ripe eggs, quality of eggs taken from the marine cages was judged to be excellent. Over-ripe eggs encountered were set aside immediately at the brood cage site for later disposal. Fertilization, experimental treatments and surface disinfection all took place at the incubation facility within hours of procuring eggs from the marine cages.

GENDER MANIPULATION

Feed-Mediated Hormone Treatment of Alevins

Of 50 specimens of the 1994 year-class that had been subjected to the food-mediated MT treatment and retained in the hatchery throughout their lives, none had satisfactory evidence of sex-reversal at the time of dissection in 1996. All of the 1997 year class of all-female eggs was produced from hermaphrodites derived from food-mediated steroid treatment of fry in 1993.

Immersion Treatment

Although steroid treatments administered in January 1995 were implemented to evaluate the efficacy of alternative-treatment protocols, insufficient space in the industry's hatchery resulted in combining of the various groups, thereby terminating efforts to evaluate relative merit of alternative techniques. As a result of common ponding in the hatchery, the most that can be said of treatments is that sex-reversed salmon were produced from the 1995 immersion treatment. In contrast to previous year-class treatments, 15% of the 1995 year-class specimens from the MDHT bath treatment were obvious hermaphrodites (Table 2). Of the masculinized females dissected in November, spermatozoa motility was excellent.

Overall, 85 specimens of the 1995 year class subjected to immersion treatment were dissected in 1997. Of the 51 specimens dissected from the marine cage at Strickland Cove in October of that year, there were only three that were obvious hermaphrodites. Motility of spermatozoa from these three specimens was poor-to-nil. This observation resulted in relocation of these potential brood salmon from the marine cage site to Ship Cove where there was a substantial freshwater layer. Dissection of specimens, randomly collected from the sex-reversed 1995 year-class in November of 1997, revealed 10 obvious hermaphrodites. Of the remaining specimens, four were normal females, all of the others revealing testes, some of which were convoluted in appearance. Of interest is the observation that genetic females often could be distinguished from phenotypic males by observing these fish swimming in the cages. Though pronounced kypes were not observed among phenotypic males, head shape when viewed from above was more elongated for the male phenotype. In the absence of definitive evidence of genetic sex, paired testes were discarded to guard against males in the 1998 year class. In contrast to results for the 1995 year class, only nine of the 60 specimens of the 1996 year class examined in 1998 were obvious hermaphrodites. There was generally inferior spermatozoa motility among seven of nine of the hermaphroditic specimens from this 1996 year class. Only two of the gonads had spermatozoa motility sufficient for reasonable certainty of fertilization. The milt of the non-motile specimens was noticeably more viscous than that of the two specimens with good motility. Homogametic milt from the two viable gonads was applied to ~90,000 Atlantic salmon eggs in support of continuing evaluation of the all-female production strategy.

Table 2. Overview, 1995 year class MDHT results.

Gonad Category	Immature		Mature	
	Number	%	Number	%
Hermaphrodite	2	2.4	13	15.3
Male	26	30.5	19	22.3
Female	11	12.9	10	11.7
Total	39	44.9	46	54.1

The "double-dipping" immersion treatment applied to alevins of the 1999 year class in February of that year was administered without notable incident. This group of potential homogametic brood salmon was retained in the hatchery only until March when hatchery management of the day decided to terminate all experimental work in the hatchery facility.

Gynogenesis

Within the confined and very damp conditions of the industry incubation facility, it proved difficult to achieve the target UV irradiation protocols. For about an hour after the UV source lamp was activated, illumination energy at the level of the sperm container fluctuated. This necessitated multiple adjustments to the height of the light fixture, adjustments that were not easily accomplished with the prototype equipment. Also, it became apparent that the supporting structure on which the UV-treatment apparatus was mounted had to be perfectly level in order to encourage uniform distribution of sperm in the glass dish. It proved difficult to control the depth of sperm in the UV-irradiation chamber, both due to surface tension and the uneven distribution of sperm within the chamber. As a result the sperm layer exposed to UV irradiation was uneven, possibly resulting in a non-uniform exposure.

Spermatozoa motility following UV treatment in 1996 appeared comparable to untreated sperm. Survival to hatch of the diploid eggs averaged 92% while the triploid eggs averaged 90.9% and eggs from the various gynogenesis treatments averaged 86.2% survival (Table 3). Variability in number of dead eggs among trays but within classification variable showed no significant difference among treatments (Kruskal-Wallis ANOVA by Ranks; $H (2, N = 72) = 5.53$, $p = 0.06$). Irrespective of interpretation of the implications of the experiment, as a result of extreme space shortages in the hatchery, all of the 1997 year class gynogens in the Bay d'Espoir hatchery were destroyed by the industry to allow reassignment of hatchery-rearing space.

Of the >100,000 salmon eggs applied to the 1997 experiment (i.e. 1998 year class for which the proscribed quartz irradiation dish was used), only about one thousand survived to the advanced alevin stage in 1998. There was no obvious pattern to survival among the three UV-C treatment intensities. Although alevins from this experiment appeared vigorous during the early post-hatch interval, they subsequently suffered a high incidence of swollen yolk-sacs reminiscent of the blue-sac condition (Davis 1946). A high percentage of these alevins died prior to yolk-sac resorption resulting in insufficient inventory remaining to justify maintaining the group integrity. Once again, the gynogens were destroyed. In comparison, survival of normal production salmon eggs to hatch in 1998 was just 51%. Survival to hatch for this year class of all-female, triploid eggs was 35%.

Table 3. Dead egg data for 1996 (1997 year class) gynogenesis experiment.

Tray / Time	Gynogenesis				Diploid		Triploid	
	E	G	I	K	D	H	F	J
1	48	75	28	85	51	82	144	45
2	414	631	336	559	101	255	306	209
3	60	25	70	75	9	57	31	37
4	31	34	50	75	70	19	30	54
5	35	30	56	68	16	20	23	52
6[a]	270	301	307	306	305	193	181	224
7	22	19	37	47	11	15	11	24
8	32	22	34	61	9	11	24	40
9	59	61	47	9	5	6	10	10
Totals	971	1198	965	1285	577	658	760	695
% Survival	87.9	85.0	87.9	83.9	92.8	91.8	90.5	91.3

[a]Time at which eggs were addled.

For the 1999 experiment at the NAFC, the three control groups had an incubation survival of 88 to 99%. Survival through incubation of the three trays of gynogens ranged from 9 to 21%. Unfortunately, tank space proved to be a problem at the NAFC and all groups of salmon had to be culled periodically as the salmon grew. Of the specimens remaining in March, 2003, the sex ratio of the control group was close to unity (48% male). Untreated gynogens were all female. MT-treated gynogens were either male or hermaphroditic (i.e. no females). None of the MDHT-treated gynogens remained at the time the experiment was terminated. Of 50 specimens of MT-treated "control" salmon sampled, all were male. Of 52 of the MDHT-treated "control" salmon sampled at the time the experiment was terminated, 41 were male and three were hermaphrodites. The remaining eight specimens appeared to be normal females. Few of the extracted gonads at the time of extraction were sufficiently mature to support interpretation of homogametic-sperm motility.

TRIPLOIDY

The simple water-bath apparatus (Fig. 5) that was used starting in 1995 for handling of eggs prior to, during, and after pressure treatment resulted in considerable improvement in survival (Table 4) for the pressure-treated eggs during the incubation cycles of the next few year classes. It was noted, during the water-hardening interval in 1995, mixed-sex diploid eggs swelled an average of 14.1%. All-female, triploid eggs swelled an average of 16.9%, a fact that needs to be taken into consideration when loading the pressure-treatment apparatus.

Table. 4. Egg survival to 50% yolk-sac absorption.

Year	Ploidy	Number	Life-stage	Survival (%)
November '94	2n	68341	eggs	
January '95		54200	alevins	79
November '94	3n	52570	eggs	
January '95		27844	alevins	53
November '95	2n	90,000	eggs	
January '96		75,861	alevins	84
November '95	3n	81000	eggs	
January '96		71793	alevins	89

The first removal of dead eggs from the incubation trays in November 1994, eight hours after fertilization, revealed early egg mortality (Table 5). The "all-female" triploids and mixed-sex diploids had a similar level of mortality after the first 24 hours. Overall egg-to-fry survival for the 1995 year class was lower for the pressure-treated groups (53%) than for the all-female, diploid line of salmon (79%). Egg-to-fry survival for the mixed-sex production line of salmon for this year class was similar (78%) to that for the all-female diploids. Further improvement was apparent for the 1996 egg take (i.e. 90% pressure-treated egg survival vs. 85% for the control group of salmon eggs).

Table 5. 1995 year class incubation performance.

Incubation Category	Ploidy								
	Triploid				Diploid				
Egg lot	1	2	3	4	1	2	3	4	5
# Eggs	15020	15020	15020	7510	15020	15020	15020	15020	8261
Initial Egg Mortality	440	517	533	546	896	774	673	362	457
# Eyed Eggs	11265	12767	12767	6008	12767	14269	14269	15020	7510
Survival to Addling (%)	75.0	85.0	85.0	80.0	85.0	95.0	95.0	100.0	90.9
Addling to Hatch Mortality	4816	4171	4516	1460	1938	2051	1964	2288	1394
Number of alevins	6449	8596	8251	4548	10829	12218	12305	12732	6116
Survival to Hatch (%)	42.9	57.2	54.9	60.6	72.1	81.3	81.9	84.8	74.0
Mean Egg to Fry Survival (%)		53.0					79.3		

First feeding mortality for triploid alevins in 1996 was 10% more than for the worst of the diploid groups. Mean weight of alevins, determined from batch weights at the time of ponding for first-feeding was similar for all groups and ranged from 0.17 to 0.19g. While

virtually all of the hatchery records of relative performance available to the authors are qualitative, there was no obvious difference in diploid and triploid parr activity or performance throughout the hatchery phase, both ploidy groups performing within normal hatchery standards.

Incubation performance of all salmonid eggs from the 1997 spawning was poor, thus giving a biased view of the quality of the triploid eggs. Survival of all-female triploid eggs was 35%, leaving only enough juvenile triploid Atlantic salmon for limited performance monitoring.

Triploid fry of the 1998 year class were maintained in the Bay d'Espoir hatchery until October 26. Unfortunately, problems with lack of hatchery space, industry financial problems, and negative perceptions from the experience of the previous year-classes, resulted in a hatchery-management decision to terminate all of the locally-produced triploid salmon performance research. However, by the time of strain destruction, quantitative comparison between strains was completely and effectively compromised by insufficient hatchery infrastructure and lack of comparable husbandry conditions.

AQUACULTURE PERFORMANCE

Husbandry

Evolving fish-health management protocols added considerably to the ability of the Bay d'Espoir salmon livestock to withstand the fish-pathogen spectrum of the area. This proved a confounding variable, adding to the challenge of interpreting the results of the gender manipulation and reproductive-control technologies applied.

According to observations by hatchery personnel, performance of the experimental group of 1995 year-class salmon parr throughout the hatchery phase was compatible with normal Bay d'Espoir hatchery standards. At the time of final hatchery sampling of the 1995 year class there was no statistical difference ($t = 1.66$, $p = 0.099$) between mean weight for the experimental group of presumed all-female triploid parr and the production line of diploid salmon. The "all-female", triploid salmon had a mean weight of 20.1g while the diploid group averaged 24.4g. At the time of the December transfer, experimental group parr averaged 23g. The mixed-sex, diploid group of Saint John River strain salmon averaged 27g live weight.

Mortality among all groups of 1994 year-class (1995 smolt) and 1995 year-class parr during the first few weeks in the estuary (Dec.-Jan.) was high. Thereafter, the mortality pattern in all of the salmon cages followed a seasonal progression that peaked with increasing water temperature, typically in late June/early July (Fig. 7). The summer of 1996 proved to be physiologically challenging when mortality during this interval was excessive. Triploid salmon were particularly hard hit, with daily mortality rising to over 1000 specimens in a 24 hour period, mortality an order of magnitude greater than for the mixed-sex, diploid control group of salmon. It proved to be a common pattern that

significant-mortality events were preceded by visible signs of *Vibrio* sp. (confirmed by laboratory analysis) and then corresponded with an outbreak of atypical furunculosis (*Aeromonas salmonicida* subspecies *nova*). Pathogen-related mortality broke out first among triploid salmon and was most pronounced in this ploidy group. Industry personnel observed that mortality among industry-production salmon broke out first and was most pronounced in those cages closest to the triploid salmon. The rest of the livestock sustained minimal losses.

In addition to the pathogen-induced mortality events, during the winter of 1996/97, it was observed that mink (*Mustela vison*) were frequent visitors to the cage site. Efforts to deter this predator were unsuccessful. During this time of winter ice cover, mink selectively attacked the triploid salmon, removing up to 24 salmon in one day.

During monthly monitoring of the experiment in 1996 through 1998, some physical abnormalities were observed in both experimental and control groups of salmon. This ranged from mild scoliosis to a low incidence of jaw deformities. In comparison to production salmon, experimental triploid salmon of the 1995 year class had a higher incidence (<1%) of jaw deformities, ranging from mild to severe. The smallest of the triploid salmon had weights similar to the predominant size category of the samples of the control groups. Interpretation of relative performance of the 1995 year class is compromised by incomplete inventory data for the industry production cage (control salmon) through parts of the ongrowing interval.

Head-on gutted weight for the triploid salmon of the 1995 year class at the time of grading in October, 1997 was 27% greater than the comparison group of mixed-sex diploids. All measures of carcass weight (Fig. 8), were greater for the triploids than for the normal industry production salmon ($t = 6.50 - 7.20$, $df = 48$, $p < 0.0001$). There was no obvious (subjective) difference in sensory quality and flesh palatability. Proximate analyses of fillet composition ($\arcsin \sqrt{proportion}$ transformed variables) suggested there was little practical difference in flesh-quality indicators between the triploid and diploid salmon (Fig. 9). Both moisture and fillet-fat data were not normally distributed ($KW > 7$, $p < 0.007$). Mann-Whitney U tests confirmed significant differences ($p < 0.007$) between diploid and triploid fillets regarding these two variables. Gut fat, fillet protein and ash values were similar between the mixed-sex, diploid and all-female, triploid salmon ($F < 1.5$, min $p = 0.23$).

Both of the "all-female" groups of this 1995 year class at time of harvest (October 1997) showed significant departures from analytical prerequisites for parametric comparison. Grilse made up 3.6% of the number of diploid salmon harvested. Small-grade diploids made up 36.4% while large-grade diploids accounted for 60% of the harvest. In comparison, small-grade triploids accounted for 45% of the harvest while large-grade triploids accounted for 55%. Large-grade triploids on average were 32% heavier than large grade diploids whereas small-grade triploids were 20% smaller on average than the small-grade diploids. Disconcerting was the fact that, on final grading of the 1995 year class, it became apparent that fully 40% of the experimental group of salmon was male. Though the overall mean weight of the triploid salmon at time of

harvest was 12% greater than the mean weight of mixed-sex, diploid salmon, weight distribution of both groups showed marked skewness and kurtosis. As well, salmon showed bimodality in their final weight distribution. Comparison of final-sample weight statistics is confounded also by heteroscedasticity (Levene $F = 24.03$, $p < 0.0001$) among the three groups (i.e. mixed-sex diploids, presumed all-female diploids and triploids). This analytical deficiency could not be resolved by logarithmic transformation (Levene $F = 18.1$, $p < 0.0001$). Kruskal-Wallis ANOVA by ranks confirmed a significant difference in live weight at time of final sampling among the three groups ($H (2, N = 306) = 13.25$ $p = 0.0013$) for the 1995 year class. Worthy of note is the observation that for this year class, the incidence of jaw deformities among harvest specimens was relatively low (1/207 diploid samples; 9/99 among triploid samples).

Triploid smolt of the 1996 year class, transferred to Margaree Cove in the spring of 1997, demonstrated alarming behaviour throughout their first few weeks in the marine cage. Activity level among all fish at the site was extreme for some two to three weeks following transfer. Multiple specimens in all cages were in the air at any one time, many bouncing off the avian-deterrent nets stretched across the top of each cage (i.e. leaps of ~1 m). Microscopic examination of water samples from the area of the cages revealed no obvious planktonic stimulus for this behaviour. There were no predators observed in the area.

Mean specimen weight at the time of first sampling for the experimental groups in 1997 after transfer to the marine cages was 43.0 g for the triploids and 48.1 g for the all-female diploids. Data for this year class were anomalous relative to other year classes in that, even at this early stage in the grow-out cycle, there was a pronounced bimodality in weights of the two groups (Fig. 10). Also, mean smolt weight for this year class was <50g as contrasted with the generally >80g mean smolt weight for the other year classes.

Growth of all salmon of the 1996 year class was poor, a situation that prevailed throughout much of 1997 and 1998. At the time of grading in October 1998, survival of the all-female triploid group was 37%. The control group of mixed-sex diploids had an overall survival to grading of 68%. Tables 6 and 7 provide an overview of sample results at the time of grading for each of the 1995 and 1996 year classes.

Table 6. Incidence of physical deformities among Atlantic salmon year classes and ploidy groups (all sampling intervals).

Ploidy	Year Class	Number of Deformities by Type					
		Scoliosis	Abnormal Jaw	Humpback	Abbreviated Operculum	Snub Nose	Total
2	1995		1				1
	1996	1					1
	1998		2				2
Totals (of n=663)		1	3				4
3	1995	1	7				8
	1996	2	19				21
	1998	1	38	1	6	1	47
Totals (of n=421)		4	64	1	6	1	76

Table 7. Year class weight statistics by industry category at harvest.

Year Class	Ploidy	Grade	Mean Weight (g)	95% Confidence Interval	
				Lower	Upper
1995	2n	grilse	2321.80	2173.87	2469.73
		small	2197.34	2013.21	2381.48
		large	3364.11	3147.31	3580.92
	3n	grilse			
		small	1762.00	1670.53	1853.48
		large	4424.77	4192.10	4657.45
1996	2n	grilse	2240.06	2121.67	2358.44
		small	2321.09	2193.71	2448.46
		large	3101.86	2970.06	3233.66
	3n	grilse			
		small	1980.37	1806.23	2154.51
		large	2951.17	2824.89	3077.46

What was apparent among triploid salmon at the time of harvest in 1998, that was observed only rarely in reproductively-competent strains, was various forms of jaw deformity ranging from eroded opercula to grossly malformed lower jaws. This was apparent also in the 1998 year class that, of the three year classes of the study, had the greatest incidence of deformities (Table 6). Of the total harvest sample of 117 triploid specimens in 2000, 17 (14.5%) had mild-to-severe deformities of the head region. Mean

weight at harvest of the 17 specimens with jaw deformities was similar ($t = 1.91$, $p = 0.058$) to that of the 100 triploid harvest salmon that had normal jaw characteristics. Variance in weights of triploid specimens with jaw deformities relative to those with normal jaw configuration was homogeneous (Levene $F(1, 115) = 1.62$, $p = 0.20$). Blood sampling confirmed that all of the Gaspé strain of salmon were in fact triploid. All of the triploid salmon were marketed in filleted form.

Results of the performance evaluation of the 1998 year class of the Gaspé strain of triploid Atlantic salmon are reported by Pepper et al. (2003). What was remarkable was that this strain of triploid Atlantic salmon totally outperformed all other groups of Atlantic salmon harvested by the Bay d'Espoir industry in 2000.

For the three year classes of performance evaluation presented above, diploid and triploid groups of salmon showed similar patterns of increase in body weight over time within years. Difference in growth performance between years was more readily apparent than differences between the diploid and triploid groups within year classes (Fig. 11), though this interpretation is confounded by incomplete inventory records for the mixed-sex, diploid (i.e. control) group of the 1995 year class and all groups of the 1996 year class (Table 8).

Survival and FCR were inferior for the triploid relative to diploid salmon of the 1995 year class. As noted above, the statistics depicted in Table 8, plus those of Table 9 are limited to the intervals for which industry records of daily feeding and inventory totally overlapped the weight-sampling intervals. This imposes an incomplete view of comparisons of diploid and triploid salmon performance. In an attempt to address this deficiency, Table 10 provides weight statistics for the groups that were available for sampling at time of harvest. As is apparent from the confidence intervals of Table 10, final mean weight at harvest for triploid salmon was significantly greater than diploid salmon for the 1995 year class (ln transformed analysis; $t = 3.55$, $p < 0.0005$, $p(\text{Levene}) = 0.93$)). This did not prevail for the 1996 year class. Regarding the 1998 year class, the final harvest weights of Table 10 are confounded by the fact that the triploid salmon were harvested on October 11 while the control group was retained until November 23. By adjusting the weight-sample data to a common harvest date of October 11 (as per Pepper et al. 2003), there was no difference in sample statistics between diploid and triploid salmon of the 1998 year class ($t = 0.68$, $p = 0.49$, $p(\text{Levene}) = 0.98$). However, harvest value of the mixed-sex, diploid salmon was compromised by the necessary removal of 310 maturing salmon that were deemed unsuitable for marketing at time of processing.

Table 8. Instantaneous rate performance comparisons for Atlantic salmon ploidy groups.

Year Class	Ploidy	Mean Weight (g)		Number		Sample		Days	G[a]	Z[a]	R[a]
		End	Start	End	Start	End	Start				
1995	2n, mixed $_{(m1)}$[b]	571.9	205.9	6570	6674	10/23/96	8/20/96	64	0.016	0.0002	0.0157
	2n, mixed $_{(m2)}$[b]	2640.4	2316.7	9470	9476	10/22/97	9/27/97	25	0.005	0.0000	0.0052
	2n, female	2549.1	101.0	7438	8002	10/21/97	7/23/96	455	0.007	0.0002	0.0069
	3n	3120.0	51.7	4449	8672	10/21/97	5/14/96	525	0.008	0.0013	0.0065
1996	2n, mixed	403.3	250.6	10192	10236	10/31/97	9/25/97	36	0.013	0.0001	0.0131
	2n, female	460.9	48.1	12199	13180	10/13/97	6/17/97	118	0.019	0.0007	0.0185
	3n	534.9	43.0	9881	11960	10/12/97	6/17/97	117	0.022	0.0016	0.0199
1998	2n, mixed	4752.1	88.0	5151	7720	11/23/00	5/24/99	549	0.007	0.0007	0.0065
	2n, female	4121.4	83.7	6476	9050	1/24/01	5/19/99	616	0.006	0.0005	0.0058
	3n	4059.2	97.8	6501	8198	10/11/00	5/19/99	511	0.007	0.0005	0.0068

[a]Instantaneous rates as per Ricker (1975).
[b]Subscript referrs to 1st and 2nd years of the marine-production cycle

Table 9. Biomass increase and food consumption for Atlantic salmon ploidy groups.

Year Class	Ploidy - Gender	Estimated Biomass (kg.)			95% Confidence on Biomass Increase		Food Dispensed (kg.)	Economic FCR			Mortality
		End	Start	Increase	Lower	Upper		Point Estimate	95% Confidence Best	95% Confidence Worst	
1995	2n mixed $_{(m1)}$[a]	3757.1	1374.5	2382.6	2159.6	2605.7	1951.0	0.82	0.75	0.90	104
	2n mixed $_{(m2)}$[a]	25004.7	21953.7	3051.0	2403.8	5698.2	1575.0	0.52	0.28	0.66	6
	2n all-female	18960.5	808.4	18152.0	13587.1	22716.9	15059.0	0.83	0.66	1.11	564
	3n	13880.9	448.1	13432.9	10456.8	16408.9	15381.5	1.15	0.94	1.47	4223[b]
1996	2n mixed	4110.9	2565.4	1545.4	1075.3	2015.6	1623.0	1.05	0.81	1.51	44
	2n all-female	5622.3	634.5	4987.8	4562.9	5412.7	4881.8	0.98	0.90	1.07	981
	3n	5285.8	514.3	4771.5	4311.2	5231.8	4584.0	0.96	0.88	1.06	2079
1998[c]	2n mixed	24939.2	679.3	24259.9	21823.4	26696.4	33159.0	1.37	1.24	1.52	2569
	2n all-female	22737.6	757.1	21980.5	17341.9	26619.2	39899.0	1.82	1.50	2.30	2574
	3n	28260.1	801.8	27458.3	24339.5	37269.0	36534.0	1.33	0.98	1.50	1697

[a] marine-ongrowing interval partitioned due to missing industry data on feeding and inventory reconciliation.

[b] mortality attributed primarily to winter vibrio.

[c] values differ slightly from those of Pepper et al. (2003) due to the previously-published values being adjusted to a common-harvest date. Present values not adjusted.

Table 10. Atlantic salmon weight statistics at time of harvest.

Year Class	Group	Sample Size	Harvest Weight (g)				
			Mean	95% Confidence		Min.	Max.
				Lower	Upper		
1995	All-female, 2n	105	2549.1	2344.6	2753.6	834.0	5054.0
	3n	99	3120.0	2877.0	3363.0	1304.0	5577.0
1996	All-female, 2n	105	2554.3	2451.1	2657.5	1020.0	4252.0
	3n	70	2465.8	2309.0	2622.5	759.0	3912.0
1998	Mixed-sex, 2n	174	4752.1	4565.4	4938.9	1925.0	8400.0
	3n	117	4059.2	3884.0	4234.4	1695.0	6040.0

DISCUSSION

Biotechnology is playing an increasingly-prominent role in development of the finfish aquaculture industry. As with other forms of animal husbandry, new approaches are sought to improve efficiency of production in order to remain competitive in a global marketplace. It has been shown that there are potential strategic and economic advantages to using late-maturing or non-reproductive salmonids for commercial aquaculture operations (Jungalwalla 1991), especially for extending the market window for fresh aquaculture product. Elimination of male salmon from aquaculture strains is one strategy that can be used to reduce early-maturation problems. Use of triploid salmon not only eliminates the early-maturation problem but also diminishes genetic concerns over potential breeding between domestic and wild fish.

Non-reproductive salmonids have been shown to be useful in avoiding the undesirable consequences of sexual maturation (i.e. growth retardation, and poor flesh/skin colour and texture). Induction of triploidy currently is the preferred method to overcome the normal reproductive cycle. Manipulation of the chromosome set is one form of biotechnology that offers possible advantages in this regard without encroaching on the controversial topic of genetically-modified organisms. Chromosome set manipulation within a species involves either imposed inheritance from one sex only or increase in the number of chromosomes from the normal diploid chromosome complement. A reliable supply of quality gametes is an essential prerequisite to application of these biotechnologies whatever the manipulation strategy.

SALMON GAMETE PROCUREMENT

Maintenance of brood stock in an estuary, in which maturing salmon have access to an upper water-column layer of lower-salinity water is one advantage of the Bay d'Espoir fjord. At time of final maturation, brood salmon in estuarine cages are able to choose salinity conditions compatible with osmotic needs for successful reproduction. Holding

broodstock in estuarine cages located close to incubation facilities has the advantage of minimizing gamete transfer time. In the Bay d'Espoir context, this has proven advantageous. Having once deduced that spermatozoa for Bay d'Espoir sex-reversed salmon were at least marginally motile, eggs from the normal brood females could be collected in a timely manner from the Bay d'Espoir cages.

GENDER MANIPULATION

Feed-Mediated Hormone Treatment of Alevins

Progress towards establishing an all-female line of salmon for Bay d'Espoir aquaculture has been tedious and far too protracted to provide practical support to industry-development needs. The Bay d'Espoir salmon aquaculture industry began experimenting with all-female lines of salmon in 1989 by subjecting newly hatched salmon alevins to a diet containing male hormones. All of the fish subjected to the treatment had to be grown to the adult stage in order to sort out those specimens that were genetic males from those females that had responded to the treatment and converted to phenotypic males. Feed-mediated attempts at sex reversal through the early 1990s were unsatisfactory. The more aggressive, actively-feeding individuals received too much of the steroid, resulting in reproductive sterilization. Those with a very low food intake during the first 50 days of feeding received insufficient hormone to mediate sex reversal. Results of these early sex-reversal experiments were disappointing, and produced very few specimens of any value to the broodstock program. This approach to developing and maintaining an all-female brood population proved to be inefficient (i.e. <5% effective) and extremely costly in terms of hatchery space, feed costs and labour.

A significant disadvantage to the food-mediated approach to gender manipulation is the commitment of hatchery rearing space to specimens that may have no future value to the aquaculture industry. Food-mediated steroid treatment has the disadvantage of highly variable dose due to inherent differences in feeding intensity among individuals. Having been exposed to the steroid treatment, should none of the treated fish produce homogametic milt, none would be marketable due to the potential for steroid residues. Of the 50 specimens of the 1994 year-class that had been subjected to the food-mediated MT treatment, none had satisfactory evidence of sex-reversal. It was apparent an alternative approach was needed

Immersion Treatment

In marked contrast to food-mediated hormone delivery, >15% of the 1995 year-class specimens from the MDHT bath treatment were obvious hermaphrodites (Table 2) even though only slightly more than half of the population was mature after two summers in the marine cage. Spermatozoa motility of the masculinized females of this year class was acceptable. While 19 of 46 mature specimens had testes and therefore may have been genetic females successfully masculanized by the treatment, these gonads were

discarded to guard against potential males in the 1998 year class. Surprisingly, only nine of the remaining 60 specimens of this year class examined in 1998 were obvious hermaphrodites. This difference may be an artefact of random sampling, though a biological basis is possible. The phenomenon of "paradoxical" sex-reversal has been observed (i.e., apparently sex-reversed fish reverting to their genetic gender) with feed-mediated androgen (17α-methyltestosterone). This may result from aromatization of excess androgen via aromatase (the enzyme that catalyzes conversion from androgens to öestrogens) or the combined effects of aromatization and inhibition of *in vivo* androgen synthesis (Piferrer and Donaldson 1994).

The paradoxical phenomenon was not likely the reason for the lower incidence of hermaphrodites among brood specimens in 1998. The sex-reversal procedure (Appendix 1), applied to the 1995 year-class from which the gonads were extracted in 1998, was based on the synthetic androgen MDHT that apparently is not subject to the same conversion to öestrogen. Also, when applied as a bath treatment, the androgen is not subject to the same digestive processes as an oral treatment. Of the 1996 year-class specimens examined in 1998, none had reverted to their genetic (i.e., female) sex. The lower incidence of hermaphrodites in the 1998 samples relative to those taken in 1997 remains unexplained, other than as a possible artefact of the sampling process and the possibility that field staff were sufficiently astute in their recognition of phenotypic males in 1997 as to remove a disproportionate number of these specimens. It is tempting also to speculate, on the basis of a 15% incidence of hermaphrodites in 1997 relative to the 9% incidence of 1998, that hermaphrodites may be easier to capture.

Lack of spermatozoa motility among seven of nine of the hermaphroditic-specimen testes in 1998 may suggest some desynchronization between the sex-reversed and normal spawners. This is not really surprising due to the fact that spermatozoa physiology typically is greatly modified in the sperm ducts of normal males. Sperm collected directly from a testis is extremely viscous relative to seamen from sperm ducts. As the sperm ducts of sex-reversed specimens typically are greatly deformed, final spermatozoa maturation is at a disadvantage among sex-reversed specimens. As noted by Billard et al. (1995), "... sperm from the testis and its quality is highly variable [among surgically removed testes]... there is no contribution of the sperm ducts to the formation of seminal fluid and the physiology of such sperm remains unknown.". Use of an extender solution (Disney et al. 1987; Palti and Thorgaard 1997), that may mimic the biochemistry of the sperm ducts or otherwise serve to prime spermatozoa motility, is a logical first step in addressing this problem. However, as noted by Billard et al. (1995), "Fish semen is viscous and difficult to mix instantaneously with diluents unless a high dilution is made or if the sample is strongly agitated, which is harmful to semen.". This is a significant warning that warrants considerable care when handling homogametic gonads and sperm samples.

Gynogenesis

Gynogenesis is one form of chromosome set manipulation that results in total maternal inheritance. It is used to produce monosex female stocks in only one generation. All-female stocks can reduce farm inefficiencies when there is a significant grilsification factor present, since for salmonids this most often is a male-centred phenomenon.

For the conditions under which our first efforts at UV irradiation of normal Atlantic salmon sperm took place, it is perhaps not surprising that egg mortality for the 1997 year class was similar in all groups and resulted in overall survival of about 90%. This is much higher than expected for the gynogenesis technique, suggesting sperm irradiation with UV may not have been effective. For this treatment, we had not yet been able to obtain the required quartz dish that would allow accurate measurement of UV energy reaching the sperm. For the 1998 year class, for which we used irradiated brook trout sperm and the proscribed quartz irradiation dish, survival to the advanced-alevin stage was lower than expected, possibly suggesting the protocol may have been effective. However, interpretations are compromised by the fact that egg quality in 1997 was poor overall and even the normal production lines demonstrated greatly diminished egg survival (51%) through incubation. Further interpretation of the results of the UV treatments for both the 1997 and 1998 year classes is not possible due to industry's decision in 1999 to terminate this research in favour of freeing up hatchery space for industry-production requirements.

Our experience with gynogenetic manipulation suggests it is preferable to utilize sperm from a related species of salmonid that does not produce viable diploid progeny when mated with Atlantic salmon. Brook trout sperm would have been desirable as a sperm source for our first gynogenesis experiment had such sperm been available. With such an interspecies cross, any eggs that survive the incubation period would have to be the result of the gynogenesis – pressure-shock process and would have to be all-female. Use of irradiated Atlantic salmon sperm with Atlantic salmon eggs forces a deferral of evaluation of success in the gynogenesis procedures until the offspring are large enough to determine ploidy status. With UV irradiation of same-species sperm, it is possible to get mixed-sex diploid, all-female diploid (gynogens), mixed-sex triploid and all-female triploid progeny in the offspring depending upon the degree of success of the UV treatment. This is obviously inefficient and an unacceptable waste of hatchery resources.

For all of the various cycles of gender manipulation under actual field conditions, we consider results to be inadequate to serve industry need for efficient use of space and resources. This interpretation, plus the industry-manager intransigence of the day regarding research projects, provided the stimulus to undertake further evaluation of the gender-manipulation protocols in the fall of 1999 at the NAFC. Given incubation survival of the control group of salmon eggs ranging from 88 to 99%, we interpret egg quality for the experiment as acceptable. That 9 to 21% of the gynogens from brook-trout irradiated sperm survived incubation suggests that the UV protocol was working and that all-female alevins had been achieved in just one generation. Likewise, the

steroid treatments undertaken at the NAFC resulted in the expected departure from the essentially 50:50 sex ratio of the control group, thereby suggesting that there is reasonable expectation of effective means to maintain homogametic brood lines given the support facilities from which to carry the required livestock inventory.

TRIPLOIDY

Aquaculture businesses are interested in pursuing technology to improve cost effectiveness and profits from salmonid farming. DFO is interested in pursuing further evaluation of methods for the production of non-maturing salmonids as a means to avoid concerns about potential long-term genetic consequences of breeding of escapees from marine cages with wild salmon of the Bay d'Espoir area.

We used the prototype pressure-shock system through the 1990s to produce experimental groups of triploid Atlantic salmon for performance evaluation under Bay d'Espoir salmon farming conditions. Image analysis of blood cells from the fish of the early experiments confirmed that triploidy had been achieved (i.e. nuclei of triploid-treatment groups had an average 33% greater area than untreated diploid cells). Subsequent use of flow cytometry confirmed that the apparatus and pressure-treatment protocol was totally reliable in producing triploid eggs at acceptable levels of incubation mortality.

The miniature upwelling-incubation system developed and applied in 1995 to facilitate egg handling prior to and after pressure shock allowed batches of eggs, equivalent to one incubation tray capacity, to be placed directly into the pressure chamber with minimal handling. This simple apparatus resulted in considerable improvement in egg survival for the 1996 and subsequent year classes (i.e. 90% pressure-treated egg survival vs. 85% for the 1996 control group of salmon eggs).

AQUACULTURE PERFORMANCE

Performance-Comparison Analyses

Performance of the 1996 year-class of triploid salmon in the marine cages is at odds with performance indicators obtained for the 1995 year-class to time-of-harvest in 1997. A higher incidence of jaw deformities among triploid harvest salmon, and poor growth performance of the 1996 year class (as indicated by the inferior harvest weights) suggest that the husbandry of triploid Atlantic salmon is fraught with many unknowns. The behaviour of all of the salmon at the Margaree Cove site immediately following transfer from the hatchery to the marine cages is the only obvious difference relative to the 1995 year-class experience and remains unexplained. The fact that this behaviour was not limited to the two cages of triploids, but was evident among all the fish at the Margaree site, suggests a common response to a common stimulus. The fact that none of the fish were feeding well at the site throughout the early summer of 1997 suggests an environmental variable that was outside the control of site attendants. Microscopic

examination of water samples from the area of the cages failed to provide any clue as to the cause of the behaviour. Whatever the stimulus, it appears the triploids were more adversely affected than the other fish at the site. This is consistent with previous observations (Graham et al. 1985; Benfey 1991) that triploids do not perform well in marginal environments. Added to the notion of environmental influence is the observed selective mink-predation event of 1996. The observation that mortality due to mink predation was limited to the cage containing triploid salmon suggests that some attribute of the triploid salmon made them more attractive to the predator. Comparison of biomass lost to mortality relative to the incidence of mortality over time illustrates the well-known fact that mortality late in the ongrowing cycle is of much greater consequence to industry economics than mortality early in the cycle. Excessive mortality of the 1995 year class of triploid salmon in the first year of the marine cycle accounted for considerably lower biomass loss than for the control group of mixed-sex diploids. Given the disparate time frames among year classes for which comprehensive data sets were available, interpretations of relative growth and feeding efficiency are compromised for the control group of the 1995 year class and all groups of the 1996 year class (i.e. limited to the first summer of the marine cycle).

It is apparent from harvest weights (Table 10) that the 1996 year class exhibited poor growth (i.e. ~2.5 kg mean harvest weight) and that variability between year classes tended to be greater than the variability between diploids and triploids. Overall, the performance of the 1996 year-class, particularly in contrast to performance of the 1995 year-class as per Pepper and Collier (1998), seems to support the contention (Benfey 1998) that husbandry requirements for triploids are not yet well defined and that triploids should be considered in the same context as new species for aquaculture development. However, analyses are compromised by the fact that smolt size of the 1996 year class at time of transfer to the marine cages was considerably less than for the other year classes.

Sampling of the 1998 year-class of triploid Atlantic salmon and control groups in the Bay d'Espoir hatchery, prior to destruction of the locally-produced triploid parr, suggested the triploid salmon, in particular those of the Gaspé strain, may have been outperforming the other hatchery salmon parr. This observation was anticipated based on the overall poor quality of eggs produced in the fall of 1997, at which time the incidence of overripe eggs was excessive and survival through incubation markedly inferior (i.e. 51% in comparison to the more usual >95%). Interpretation of relative performance of the imported (Gaspé) and locally-produced triploid salmon through the hatchery phase for the 1998 year class must be tempered with the fact that the imported strain experienced hatchery density conditions of only 26 $kg \cdot m^{-3}$ while the locally-produced strain was confined in hatchery tanks where density reached 68 $kg \cdot m^{-3}$. Superior performance of the Gaspé strain relative to the industry-standard, mixed-sex diploid strain was confirmed during the marine-growth interval of the experiment (Pepper et al. 2003).

As was apparent from the observation that 40% of the 1995 year class of supposedly all-female triploid salmon was in fact male, the first of the MDHT, alevin-immersion treatments was not satisfactory. Despite the fact that the harvest sizes attained

by the triploid salmon averaged 12% greater than the mean weight of the control group, and all of the triploid salmon were marketable, any incidence of males among commercially-grown triploid salmon is unacceptable (as per Thorgaard and Allen 1987). It was this deficiency that required evaluation of the alternative (gynogenesis) approach to gender-manipulation started in 1996.

Proximate analyses of diploid and triploid salmon fillets from the 1997 pre-harvest grading suggest no real differences in fillet quality. Similar values of % gut fat (Fig. 9) for this harvest, especially around the pyloric caeca, suggest similar physiological energy reserves for both diploid and triploid salmon. A 27% improvement in mean final carcass size of the triploid relative to the diploid control groups of this 1995 year class of Atlantic salmon provided sufficient stimulus at the time to continue with this reproductive-control technology. Importation of the trial group of triploid Gaspé-strain salmon from Cascade Aquafarms (State of Washington), for comparative-performance evaluation against the new year-class of triploid Atlantic salmon produced in St. Alban's in November 1997, provided a very practical demonstration of the potential advantages of application of this technology. An unfortunate confounding variable to industry's perception of the performance of locally-produced relative to imported triploid Atlantic salmon was the inferior quality of all Bay d'Espoir eggs in 1997. It remains a possibility that the decision to abandon the reproductive-technology research was influenced more by the consequences of poor egg quality in 1997 and industry financial concerns regarding use of hatchery space than to actual performance characteristics of triploid salmon.

While the greater incidence of jaw deformities observed among triploid Atlantic salmon would be undesirable in a head-on-gutted market, the only significant difference we found for fillets was greater weight per fillet for triploid Atlantic salmon. This is at odds with experience from elsewhere in the Maritimes (pers. comm. Dr. Larry Hammell, AVC, Charlottetown, PE) in which mortality among salmon with jaw deformities was roughly double and final harvest weight significantly lower for non-deformed salmon.

Proximate analyses of the present study failed to reveal differences between diploid and triploid fillets that might influence consumer acceptance. However, variability in production characteristics of triploid salmon from year to year proved to be a concern to the salmon-farming industry. Amid cash-flow challenges, insufficient hatchery facilities to carry experimental lines, and the industry interpretation of greater susceptibility of triploid salmon to the fish-pathogen spectrum of the Bay d'Espoir estuarine fjord, the industry decision to terminate the reproductive-technologies research was inevitable. Based on a decade of industry contribution to the various components of the reproductive-technologies experiments, industry intent was proactive. Unfortunately, realities of industry economics and the vagaries of market conditions do not provide a stable base from which to advocate industry-based, longer-term research commitments. Ironically, developing industries are those most in need of new knowledge towards economic viability, yet are those least likely to afford the required infrastructure or be able to support long-term cost commitments. Termination of the reproductive-technologies research of this report is due largely to these economic considerations.

Overall the main conclusion, based on results of our experience with production of all-female triploid Atlantic salmon from local stocks, and evaluation of the Gaspé strain of triploid salmon (Pepper et al. 2003) is that triploid Atlantic salmon, produced from a superior-performing aquaculture strain, can result in performance advantages. The range of environmental conditions under which superior performance can be expected remains unresolved. Given the extreme and competing demands on Newfoundland's fledgling aquaculture businesses, if these knowledge deficiencies are to be addressed, it is unrealistic to expect the necessary continuity of rigorous experimental design from industry workers. Research endeavours in support of sustainable Canadian aquaculture require true partnering between the industry developers and the public beneficiaries of viable Canadian industry.

LACK OF BROODSTOCK FACILITIES

A common theme throughout the decade of these reproductive-technologies experiments, one that is reflected in this report, is that of having to abandon research in progress due to lack of facilities in which to carry the various experimental groups. What is taken from this experience is that research, based entirely on industry production facilities, is at the mercy of industry financial obligations. In matters pertaining to brood lines and performance characteristics, species generation time dictates time lines of 15 to 20 years. It is apparent that the present Newfoundland and Labrador aquaculture industry simply does not have the financial capacity or stability to underwrite research covering such time frames. In light of experience gleaned from salmonid aquaculture in other parts of the world, the benefits of broodstock research, research that has been performed with considerable government commitment, has proven beneficial and has attracted considerable international interest in gaining access to these selected brood stocks. If the Canadian aquaculture industry is to achieve competitive advantage in a global marketplace, public investment in broodstock facilities is essential.

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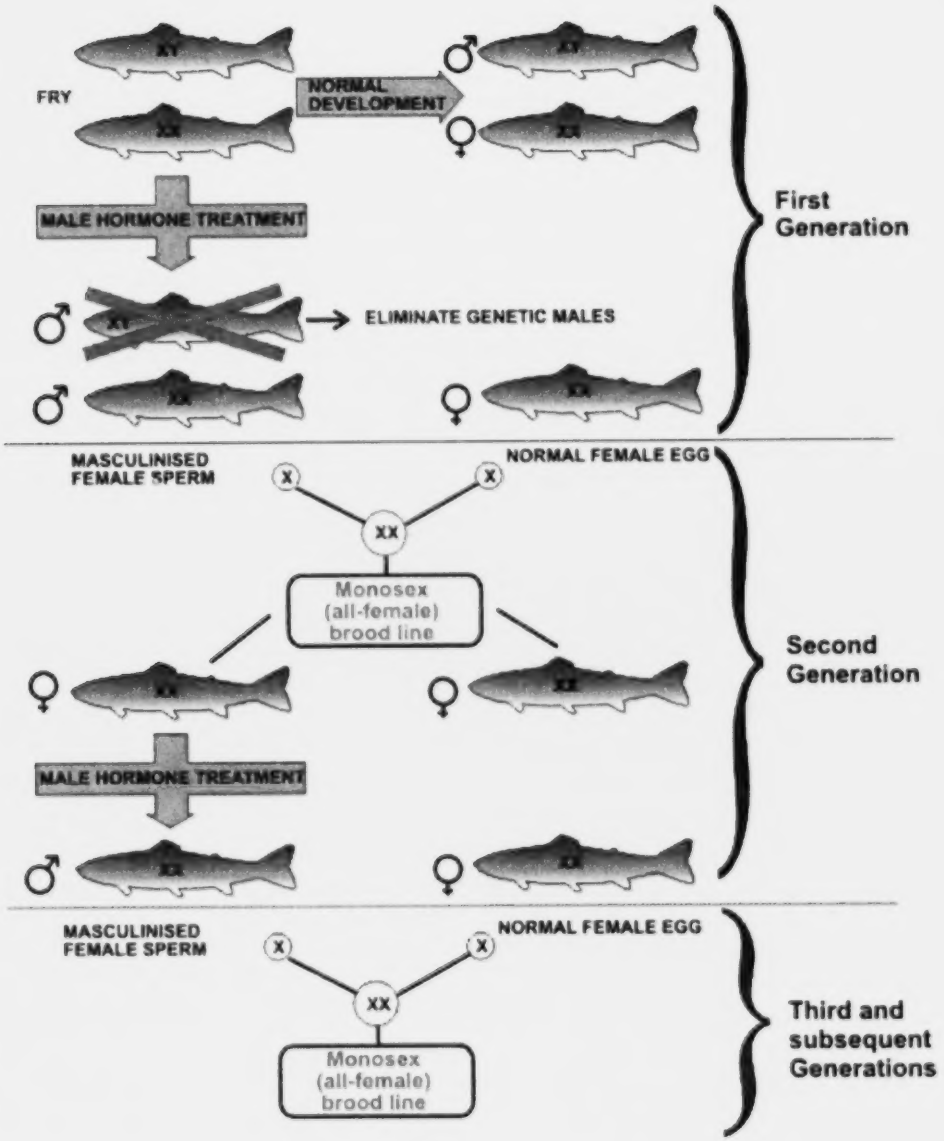


Figure 1. Monosex production strategy based on treatment with male hormone.

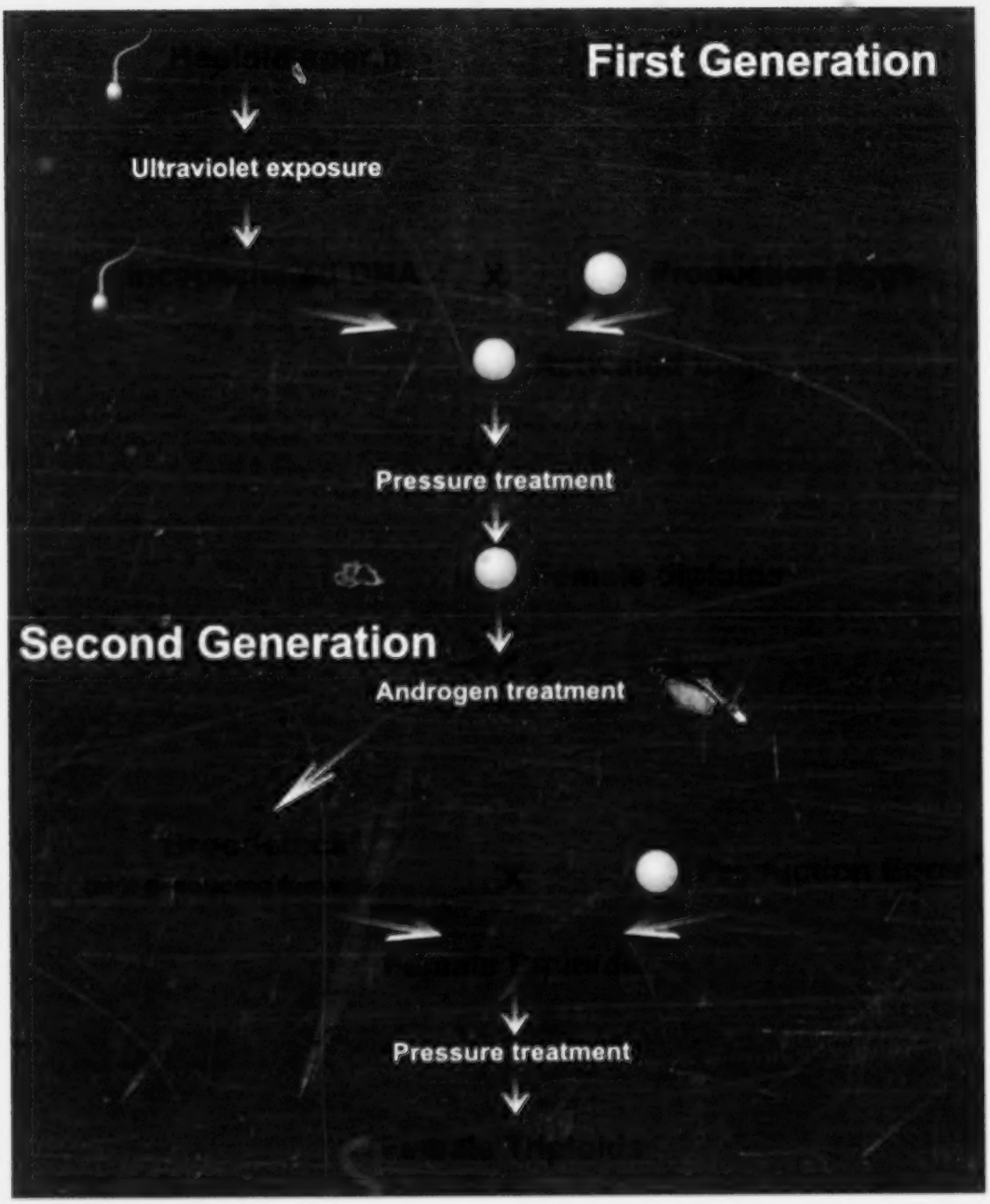
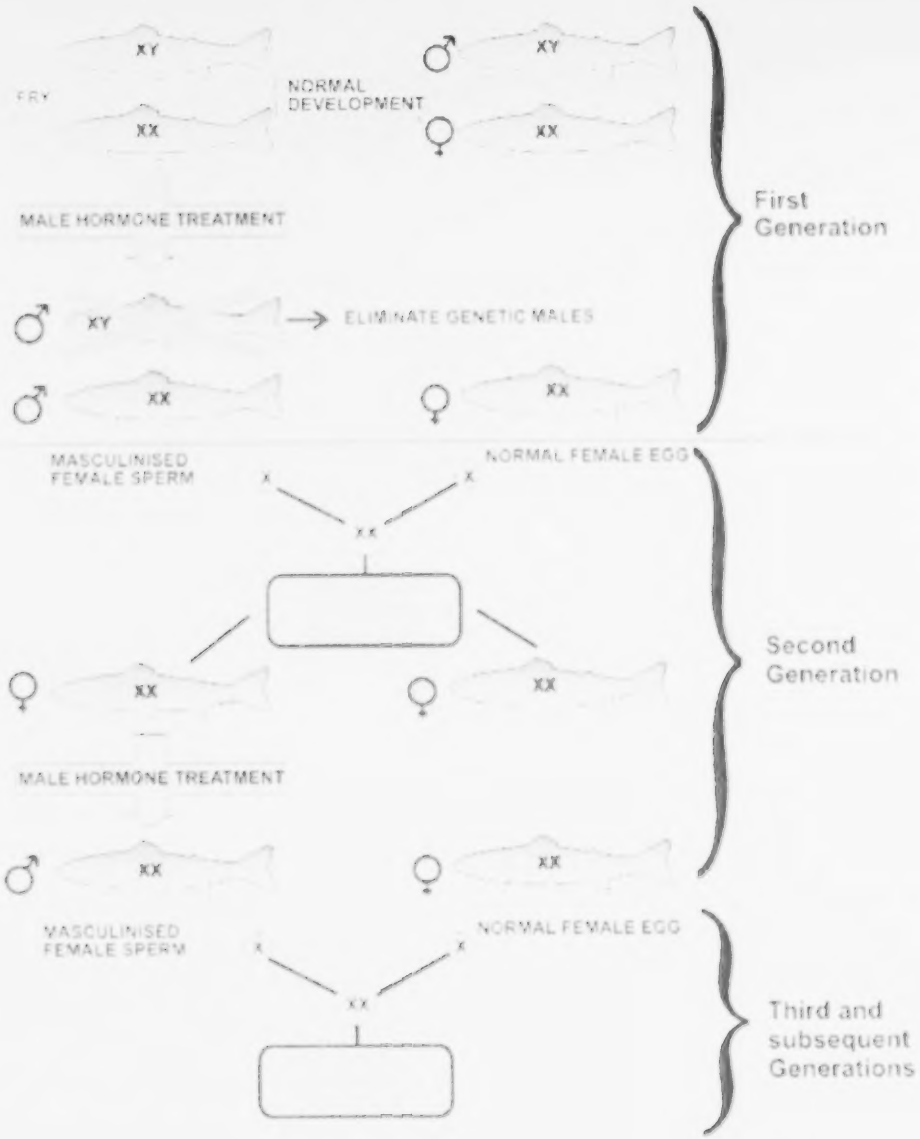


Figure 2. Protocol for gynogenic approach to producing monosex and triploid salmon.



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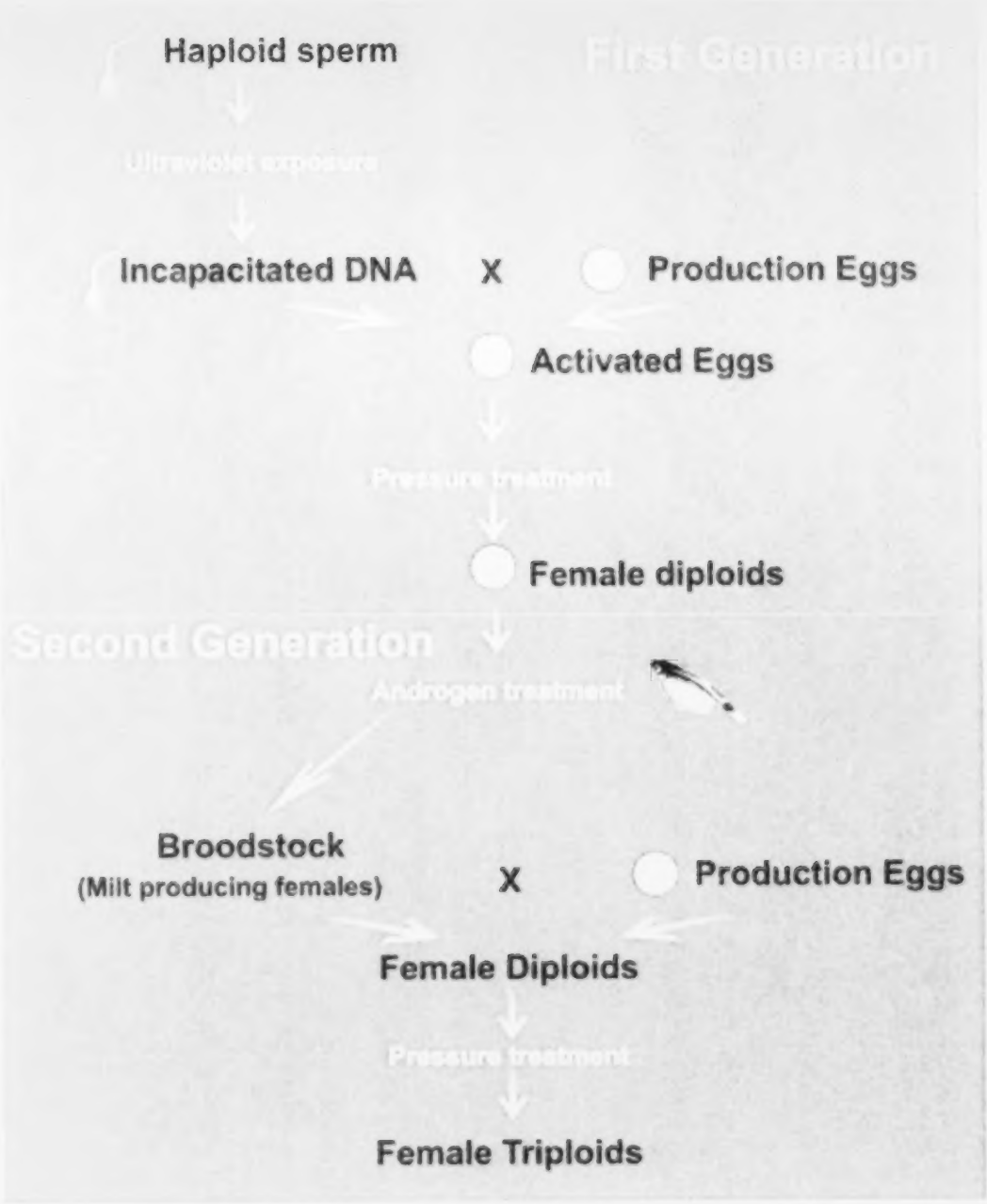

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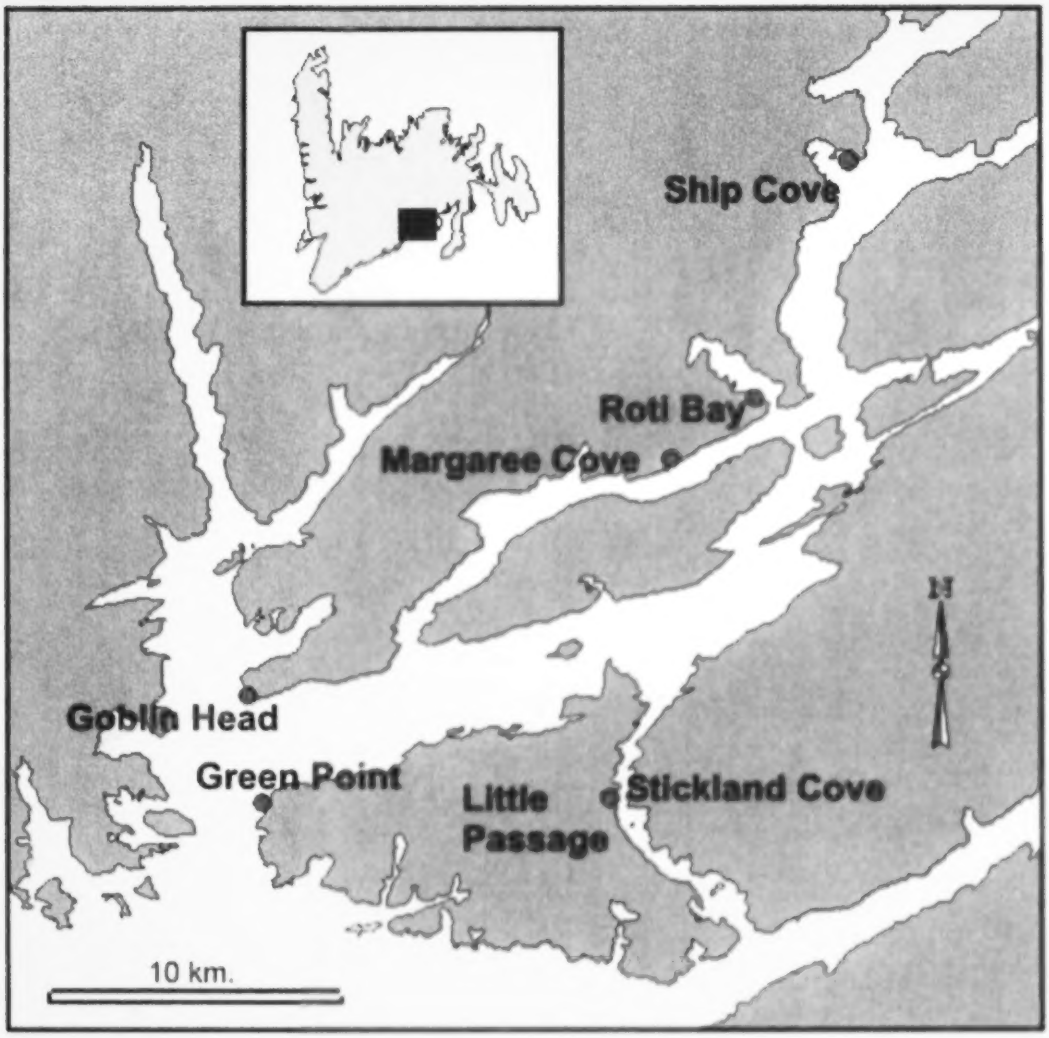

Figure 3. Bay d'Espoir aquaculture sites and geographic features.

Figure 4. Hermaphroditic Atlantic salmon.

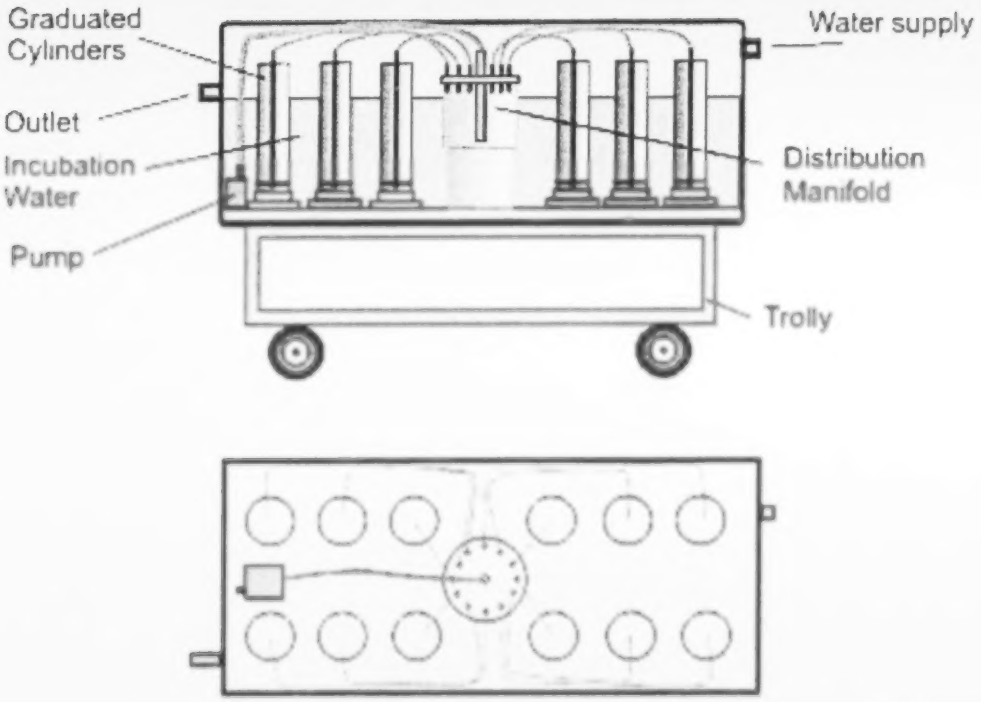

Figure 5. Upwelling incubation apparatus for handling Atlantic salmon eggs immediately post fertilization.

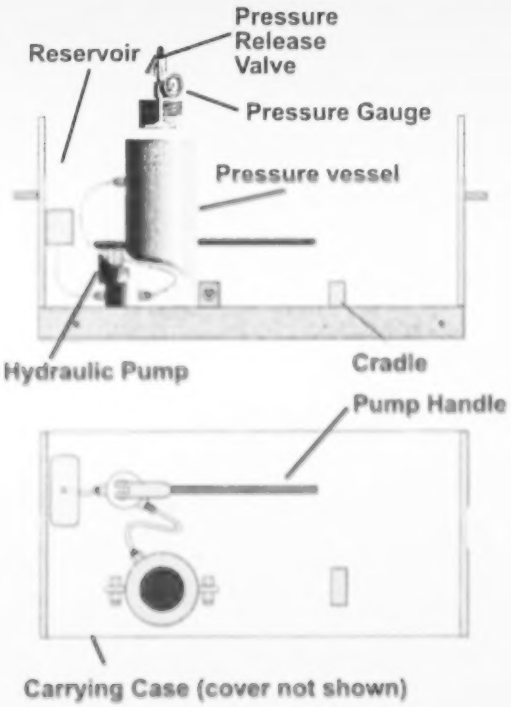

Figure 6. Pressure apparatus for producing triploid Atlantic salmon zygotes.

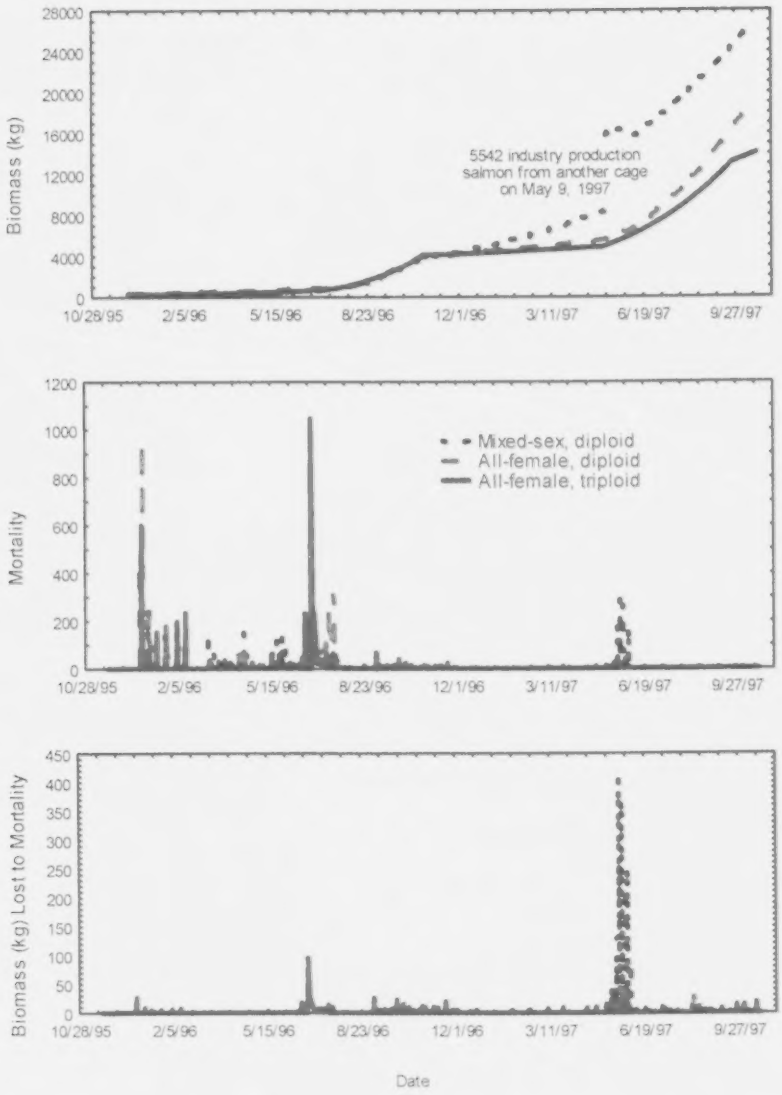

Figure 7. Biomass gains vs. mortality losses for 1995 year class.

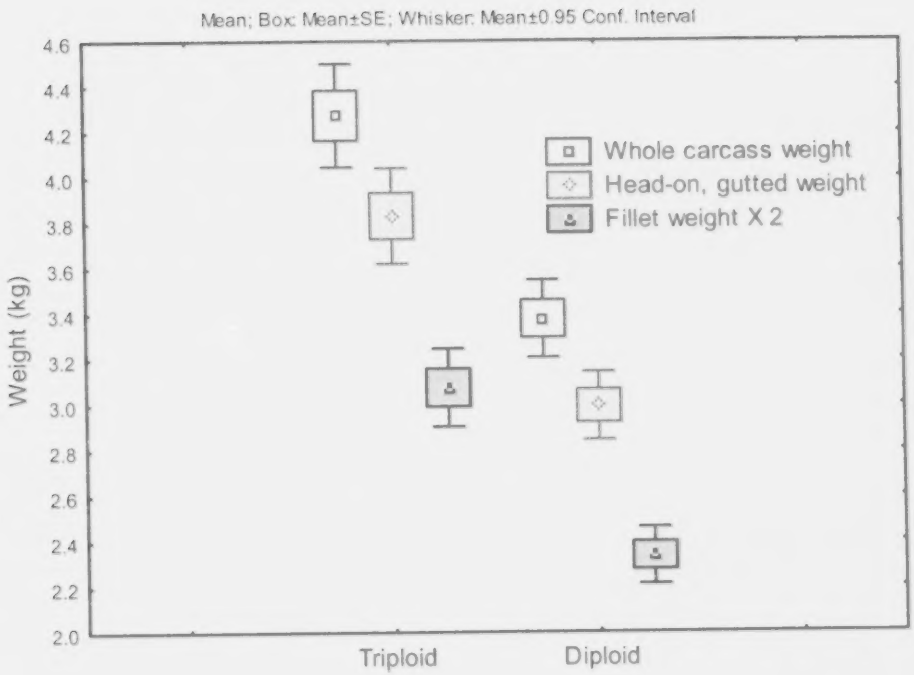

Figure 8. Comparison of Atlantic salmon carcass weights at time of pre-harvest grading of the 1995 year class.

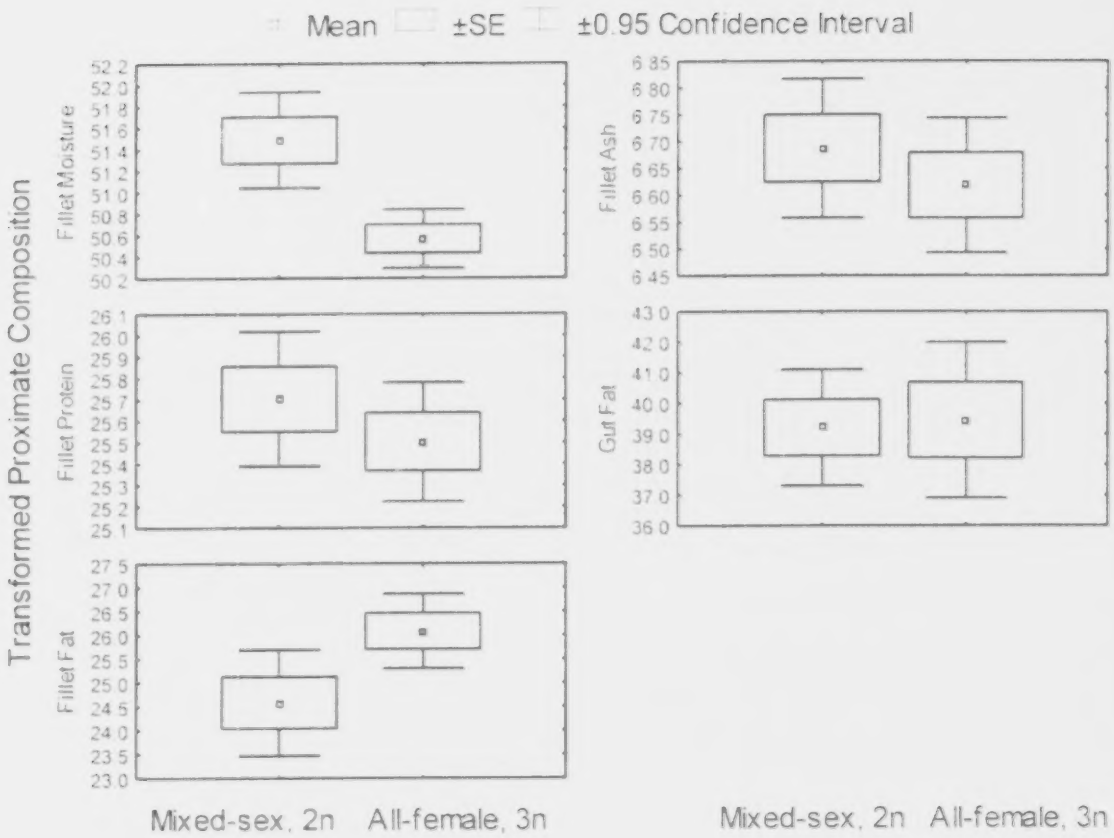

Figure 9. Comparison of proximate analyses (arcsin $\sqrt{proportion}$ transformed) of diploid and triploid salmon fillets.

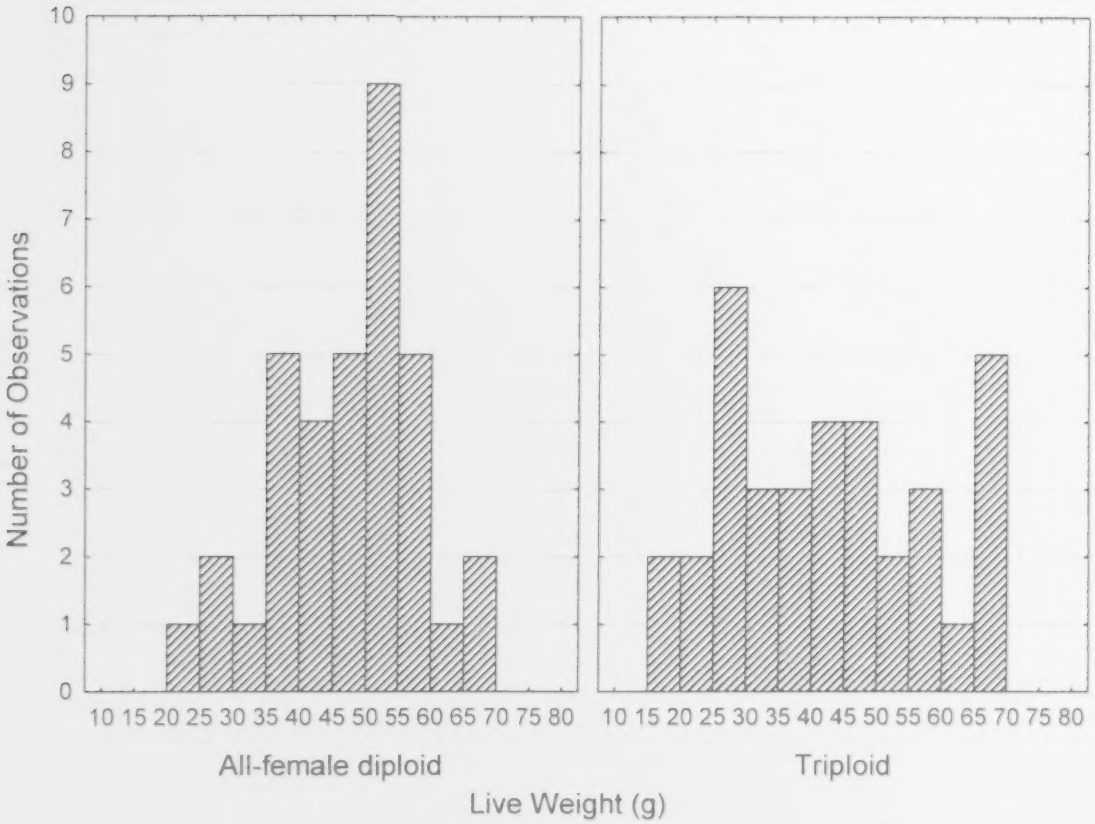

Figure 10. Non-normal distribution of Atlantic salmon parr weights at transfer to marine cages for the 1996 year class.

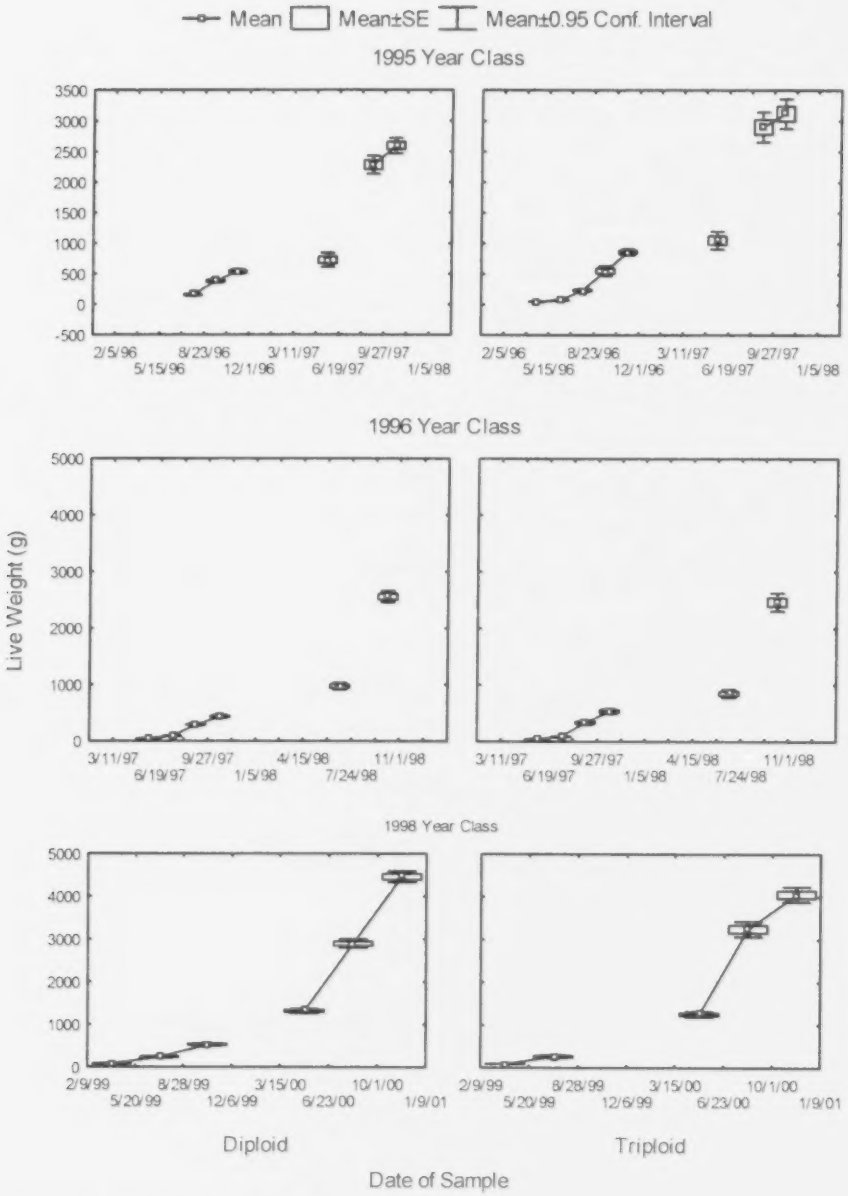

Figure 11. Temporal weight sampling data for diploid and triploid Atlantic salmon.

Appendix 1. Immersion treatment for indirect feminization of Atlantic salmon.

The following technique is based on an unpublished report by I.I. Solar and E.M. Donaldson, 1994 as per a personal communication with Dr. Donaldson. Treatment timing identified as "hatch" + 7-14 days @10°C.

In the context of Bay d'Espoir aquaculture, a batch for steroid treatment consisted of ~5,000 alevins. Alevins were subjected to a bath of 17α-methyldihydrotestosterone (MDHT) @2,000 µg/litre @250 alevins per litre. The bath treatment is administered at 140C° days after the date of modal hatch (i.e. at an incubation temperature of 8°C, the bath would be administered at 18 days [140/8 = 17.5] after hatch). Alevins are immersed in the steroid bath for 1200C° minutes (i.e. at an incubation temperature of 8°C, the bath would be administered for [1200/8 = 150 minutes or 2.5 hours].

Equipment

1 2 litre graduated cylinder
2 1 plastic tote tray (~80 cm x 50 cm)
3 1 vertical-stack incubation tray
4 2 Hagen Battery air pumps
5 air hose
6 latex examination gloves
7 2 air stones
8 1 x 2 litre measuring "cup" (must have large bottom area to volume ratio)
9 aquarium dip net
10 40,000 µg MDHT (pre-weighed)
11 ~5ml Ethanol
12 5,000 alevins of mixed parentage
13 timer with alarm to monitor measured interval of ~2.5 hr.

Protocol

Optional

Determine actual volumetric displacement of alevins. If there is reason to expect the alevins are either significantly larger or smaller than usual, it may be worth while to do a few of 100 ml volumetric displacement counts. However, even though the alevins should have about 50% yolk-sac absorption at 140C° days after the date of modal hatch, yolk-sacs still will be extremely delicate. A value of 820 alevins·100^{-1} ml displacement may be used as a guide.

Procedure

1. Set up a water bath in the water hardening table.
2. Put the tote tray in the water bath.
3. Place 10 litres of incubation water in the tote tray.
4. Make sure to wear the latex examination gloves during all of the subsequent steps. Add ~5 ml of ethanol to the pre-weighed vial of 40,000 µg MDHT. Make sure the MDHT crystals are thoroughly dissolved in the ethanol.
5. Pour the ethanol/MDHT solution into the tote tray.
6. Add an additional 10 litres of incubation water and assure mixing of the solution.
7. Put air stones in the tote tray and allow water to bubble for at least five minutes.
8. Place an empty incubation tray in the water hardening table and fill with incubation water.
9. Place exactly 1000 ml of incubation water in the measuring "cup".
10. Using the aquarium dip net, very carefully scoop up a few hundred alevins from an incubation tray. Drain excess water from the net for a few seconds, then place alevins directly into the water in the measuring cup. Note the water level in the measuring vessel.
11. Repeat the process until the water level in the vessel is just slightly more than 1600 ml (average volumetric displacement should be ~820 alevins/100 ml).
12. Carefully place the 600+ ml. volume displacement of alevins into the incubation basket in the incubation tray.
13. Remove the basket from the incubation tray, allow to drain for about 10 seconds, then carefully place the incubation basket into the MDHT bath. Immediately start the timer. Do not cover the incubation basket with its mesh lid as this will hamper air bubbling through the basket.
14. Check the bath periodically to assure the air stones are working properly. Gently move the incubation basket up and down within the tote tray a few mm periodically to assure water exchange between the bath and the inside of the basket to keep the hormone distributed to the alevins (be careful that the alevins do not escape to the bath).
15. Shortly before the end of the bath interval, start water flowing to the back of the incubation tray to assure the tray is full of fresh water at the same temperature as the treatment bath water.
16. At the end of the predetermined bath interval, turn off the water flow to the incubation tray and allow the level in the tray to stabilize. Remove the incubation basket from the hormone bath and allow to drain for ~10 seconds. Place the basket back into the incubation tray, cover with the mesh lid, and start a gentle water flow to the back of the tray while it is still on top of the water hardening table. Allow the tray to flush for at least 10 minutes.
17. Place the incubation tray into the incubation stack, being careful to record the stack number, alevin lot designation and position of the tray within the stack.
18. Rinse all treatment equipment and remove from the incubation facility into appropriate storage.
19. Check treated alevins the day after treatment to confirm a low level of post-treatment mortality.